Report to
Environmental Protection Authority
for

Activities under ERMA 200223

AgResearch Ltd

For the 10 years ending
15th April 2020

Submitted by Tim Hale
Operator / Manager
AgResearch Ruakura
Animal Containment Facility

Contents

Summary of Activities for the period 15th April 2010 to 15th April 2020

This summary provides the information required by control 11 (Annual reporting) of the HSNO Act approval ERMA200223.

Please note that information provided under 'Summary of Science Activities for the 10-year period to 15th April 2020', section a), point 9. **Genetically engineered goats capable of producing female only offspring** and section c), point 9. **Genetically engineered goats capable of producing female only offspring**, is confidential and not suitable for public dissemination. The information is marked as confidential below.

Outdoor Development Activities

All outdoor development activities being carried out within the Animal Containment Facility at Ruakura comply with the requirements of the ERMA200223 approval.

Cattle, still alive at the end of the reporting period have now only been developed and maintained under the ERMA200223 approval.

Goat development and maintenance activities now only involve animals developed under the ERMA200223 approval.

Cattle, goat and sheep activities, other than the maintenance or growing of animals, have been flushing eggs from fertile animals, kidding of goats, lambing of recipient ewes and the transfer of embryos to recipient animals. Semen has been collected from Bulls, Bucks and Rams for analysis or storage for future use.

All genetic modifications introduced into cattle, goat and sheep fall within the approved organism description for the ERMA200223 approval and are for either the production of human therapeutic proteins, or for the study of gene function.

All activities involving experimental animals have been undertaken with the approval of the Ruakura Animal Ethics Committee.

Further details on development activities are provided within the respective Science, Management and Ethics reports sections below.

Unforeseen adverse effects resulting from the genetic modifications

There have been no unforeseen adverse effects identified since ERMA200223 was approved.

Iwi liaison group relationship development and management activities

The ERMA200223 Liaison Group has still not officially met since December 2011.

As advised in previous annual reports, at the request of a group of Ngati - Wairere elders the Liaison meetings were put on hold, while representation and membership of the Liaison group was discussed within the Hapu.

Following some correspondence and individual contact, this group of Ngati - Wairere elders was invited and did visit Ruakura in October 2012 and a process to progress representation was discussed. Unfortunately, due to circumstances outside of AgResearch influence and despite further attempts, no progress has been made in resolving this directly to date.

There has again been some informal contact with original monitoring group members and regular contact with Tainui Group Holdings on their development activities for Ruakura.

AgResearch's Manager Māori - Strategy and Engagement who has local affiliations and his team are still working to build a relationship with Ngati - Wairere for Liaison Group and other Ruakura initiatives of interest to Ngati -Wairere and Tainui.

Members of the AgResearch Maori and Animal Science teams did meet in November 2019 with representatives from Waikato Tainui and Maniapoto, principally to discuss a new area of GM animal science. These discussions did include a brief summary of current and previous research projects and consultation history, along with the goal of development of a long-term relationship for constructive consultation. A second meeting was planned for March 2020, but postponed due to Covid -19.

Further information is provided as part of the summary of science activities in the section entitled 'b) any adverse effects of the organisms that have occurred, including any effects which relate to the matters described in section 6(d) and the principles of the Treaty of Waitangi (Te Tiriti o Waitangi)' below.

Additional Supporting Information

The following reports are supporting information provided to expand on previous summaries as part of the annual reporting and provide evidence of wider compliance with ERMA200223 Controls and MAF/ERMA New Zealand Standard '*Containment Standard for Field Testing of Farm Animals'.*
This additional supporting information is also provided to enable equivalence to the previous annual reporting for the inactive GMF98009 approvals.

Summary of Science Activities for the 10-year period to 15th April 2020

This summary provides the information required by control 12 (10year reporting) of the HSNO Act approval ERMA200223.

a) any progress that the approval holder has achieved towards completion of the proof-of-concept research

The EPA approval (ERMA200223) covers not a single proof-of-concept study but a range of projects in different species investigating the production of high value proteins and endogenous gene function for various applications. Completion of proof-of-concept can be achieved but considering the relative long generation time of livestock, the dependency on long-term funding, and the comprehensive validation required for the products or value of the genetically engineered animals to complete the proof-of-concept, it is not unexpected that completion of our studies fall outside the ten year reporting period. Hence, substantial progress towards proof-of-concept has been made with all our projects but remain in the research and development stage and have not yet progressed to commercial applications.

1. Cattle producing high casein milk

Cattle were characterised over five generations for stability of the genotype, casein overexpression phenotype and any long-term safety and health issues. Stability of phenotype and genotype was confirmed and the impact of the additional casein genes on milk composition was analysed in further detail. No evidence was found for unexpected adverse effects as a result of introducing extra casein genes. Relevant results were published as two major peer reviewed research articles in international science journals (Brophy et al., 2003; Laible et al., 2016[b]). The transgenic lines are no longer represented as live animals, but the genetics have been preserved as cryopreserved somatic cells and sperm. Additional analyses of the high casein milk are planned as part of an international collaboration to further elucidate the effects on the functional attributes of the milk.

2. Overexpression of recombinant human myelin basic protein (rhMBP) in milk

After initially demonstrating the successful production of the transgene-encoded rhMBP and confirming stability of genotype and phenotype over three generations, the main focus has been on the isolation of

rhMBP from milk, its functional characteristics and potential use as a therapeutic treatment option for multiple sclerosis (MS). Relevant results were published in five major peer reviewed research articles in international science journals (Al-Ghobashy et al., 2009; Al-Ghobashy et al., 2010; Al-Ghobashy et al., 2011; Al-Ghobashy et al., 2013; Al-Ghobashy et al., 2017). The latest article describes the potential of the cow-produced rhMBP as a therapeutic vaccine for MS. The transgenic lines are no longer represented as live animals, but the genetics have been preserved as cryopreserved somatic cells and sperm. Further analysis of the functional properties and potential as therapeutic agent of rhMBP produced in the milk of the transgenic cattle are planned as part of an ongoing international collaboration.

3. Production of the anti-cancer antibody cetuximab in the milk of transgenic goats

Several lines of transgenic goats were produced and characterised for their genotype and production levels of the recombinant cetuximab antibody in milk. Two goat lines with suitable cetuximab production levels were selected and characterised in detail for the number of integrated transgene copies, integrity of all transgene copies, the chromosomal site of integration, stability of genotype and phenotype over multiple generations. The recombinant antibody was purified from milk and characterised for a range of functional attributes including in vivo half-life, glycosylation, binding specificity for its cognate target EGFR, inhibition of EGFR signalling, antibody-dependent cell-dependent cytotoxicity and inhibition of tumour growths in a mouse model. The goat-produced antibody was shown to have similar anti-tumour efficacy as the originator drug but an improved glycosylation profile and enhanced antibody-dependent cellular cytotoxicity This shows that the transgenic goat production system is an effective way to produce therapeutic antibodies. The main results were summarised in two manuscripts that were deposited as pre-print at bioRxiv (Laible et al., 2020; Wang et al., 2020) and then submitted to international peer-reviewed scientific journals. One article has been accepted for publication; the other is in final stages of revision after peer review. The unique genetics have been preserved as cryopreserved somatic cells and sperm. Additional pre-clinical data will be required to progress to clinical trials. Live goats are maintained to facilitate breeding and provide access to the antibody for further testing.

4. Changing milk composition by RNA interference

The endogenous bovine milk protein beta-lactoglobulin (BLG) has no human equivalent and its biological function is poorly understood. It is recognised as a major cows' milk allergen that can cause, even severe, allergic reactions following consumption of cows' milk. To reduce or eliminate BLG in cows' milk we introduced a transgene for the mammary gland expression of a microRNA targeting the BLG gene into the bovine genome. A cow, with the transgene, produced milk where BLG was no longer detectable. It showed the suitability of the technology (RNA interference) to essentially eliminate a known allergen from cows' milk. The results were published in a prestigious international science journal (Jabed et al., 2012). In the founder animal, copies of the transgene were integrated on three different chromosomes which segregated in the next generation according to expected patterns. Each insertion site was stably transmitted and only one transgene insertion site was required for effectively abolishing the production of BLG. Unexpectedly, the founder cow was born without a tail. Tailessness is a known congenital defect in cattle where the underlying cause is not fully understood. All offspring from the original founder cow had tails excluding the genetic modification as the cause for the missing tail. Two foetuses generated from cells derived from the tailless founder animal, and thus genetically identical with the founder, had tails. This further excluded the presence of a mutation in the individual cell used to generate the founder cow. Rather, it suggested an epigenetic error during nuclear reprogramming involved in the generation of the founder cow as the cause for the missing tail. These results have been published as a peer-reviewed article in an international science journal (Wagner et al., 2017). The valuable genetics have been preserved as cryopreserved somatic cells and sperm. Live cows are maintained to provide access to milk and testing of the functional properties of the transgene-derived microRNA in milk as part of an international collaboration.

5. Genome editing cattle for the production of hypoallergenic milk

Complete prevention of *BLG* production can only be achieved by disrupting the gene responsible for *BLG* production. We disrupted the *BLG* gene with the introduction of a 9bp deletion directly in bovine embryos. Two calves from these embryos had essentially fully converted genotypes while a third one possessed some cells (10-15%) still capable of producing *BLG*. The latter is a disadvantage of the embryo-mediated approach that can simply be resolved by breeding into the next generation. The disadvantage needs to be balanced against the greater efficiencies of producing live animals. The genotypes were analysed in detail and no evidence for any unintended off-target mutations was found. The milk showed total absence of *BLG* for the fully converted genotype and small amount of a smaller *BLG* variant derived from the small proportion of still functional genes. Overall, it demonstrated that a complete, precise disruption can be achieved directly in embryos and that it is possible to safely eliminate a major cows' milk allergen with this approach. The results have been published in two peer-reviewed articles in an international science journal (Wei et al., 2015; Wei et al., 2018). The genetics have been preserved as cryopreserved somatic cells and sperm. A single female cow is being maintained to enable further studies into the mechanism of the introduction of genetic modifications in embryos and provide access to milk for further studies.

6. Germline complementation in sheep

Artificial insemination (AI) drives genetic gain in dairy cattle. However, sheep AI is invasive, labour-intensive and costly, resulting in slow genetic improvement by natural mating. The AI-on-hooves concept combines practicality of natural mating with genetic impact of AI. We have investigated the function of the spermatogonial stem cell-specific genes *DAZL* and *NANOS2* in order to ablate the male germline in rams. Using a combination of gene editing the targets and somatic cell cloning, we generated sterile *DAZL* knockout neonate rams. For proof-of-concept, we have successfully produced viable cloned sheep from the following *NANOS2* genotypes: i) elite commercial wild-type rams of the short-tail, easy-care trait combination, ii) sterile *NANOS2* knockout rams, iii) chimaeric 'absolute transmitters' carrying the germline of a genetically elite wild-type donor ram in an otherwise *NANOS2* knockout animal, iv) fertile heterozygous male hosts and v) fertile homozygous female hosts. The latter two genotypes are required to continuously supply sterile host embryos for germline complementation, providing a long-term supply of sterile host embryos via in vitro breeding. This work establishes a robust genome editing platform, underpinned by sophisticated assisted reproductive technologies, for advanced sheep breeding in NZ. It allows setting up a novel breeding approach to generate absolute transmitters without using somatic cloning. We are the first group in NZ to gene edit sheep and our work was published in several abstracts and has been deposited on preprint servers (McLean et al, 2019).

7. Kidney complementation in immune-compatible sheep

Farm animals have been proposed as hosts to grow human organs for xenotransplantation. This concept involves genetically disrupting target organ development in the livestock host, followed by complementation with human cells that populate the vacant niche and generate the transplantable organ. To generate anephric, immune-compatible hosts for organ complementation, we targeted three genes for functional evaluation: a putative master regulator for kidney development, spalt-like transcription factor 1 (*SALL1*), as well as two major xenoantigens involved in hyperacute immune reaction, galactose-α(1,3)-galactose (α-Gal) and N-glycolylneuraminic acid (Neu5Gc). We chose sheep as the host species because they have a similar kidney anatomy to humans but are perceived as culturally more acceptable organ donors than pigs.

We simultaneously knocked out *SALL1* and the genes underlying α-Gal and Neu5Gc formation, α(1,3)galactosyl transferase (*GGTA*) and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (*CMAH*), respectively. Ovine fetal fibroblasts (OFFs) were transiently transfected with three plasmids, each for a different target gene. One triple knockout (TKO) cell strain, with no observed mutations at the top three predicted off-target sites for each gene, was used for somatic cell cloning. For complementation, male TKO host embryos were aggregated with cloned female double KO (DKO) donor embryos

(*SALL1*$^{+/+}$/*GGTA*$^{-/-}$/*CMAH*$^{-/-}$), carrying a red fluorescent protein (RFP) reporter to trace the donor genotype (*SALL1*$^{+/+}$↔*SALL*$^{-/-}$ chimaeras). Non-chimaeric cloned DKO embryos with and without RFP served as wild-type controls for normal kidney development. Following embryo transfer into surrogate ewes, TKO fetuses were recovered that showed variable renal hypoplasia, which was partially rescued in chimaeras. Overall, this suggests that the kidney niche in *SALL*$^{-/-}$ males is vacant but intact and can be partially rescued by embryo complementation.

8. Improved bovine cell reprogramming by epigenetic modulators

Somatic cell transfer (SCT) cloning is a unique reproductive technology that enables the production of animals with complex genetic modifications, which are required for therapeutic and agricultural applications. A reliable, safe SCT procedure offers unparalleled advantages for the production of multi-gene edited animals. Therefore, a systematic approach that aims to reduce the SCT-related animal pathologies is needed. We have been improving SCT efficiency by systematically refining this multi-step technology to reduce the incidence of the health issues beyond mid-gestation. The most promising refinement is the use of genome-wide modulators to remove epigenetic barriers and increase reprogramming efficiency, namely enzyme-mediated removal of histone marks. We discovered that histone (H) lysine (K) tri-methylation (me3) marks (H3K9me3) pose a major epigenetic barrier in SCT cloning (Wei, Antony et al, 2017). Overexpressing the lysine demethylase Kdm4b in donor cells correlated with improved development of cloned mouse embryos (Wei, Antony et al, 2017; Antony, Oback et al, 2013). Our finding was confirmed by other groups, using different Kdm4 isoforms and extended to human, monkeys and sheep. We evaluated the function of this gene in cattle. Even though Kdm4b expression in donor cells did so far not improve cloning efficiency to term, it led to a derestricted genome with greater *in vitro* reprogrammability (Meng et al, 2020). This work generated a single transgenic cow that conditionally overexpresses *KDM4B*, providing a new transgenic animal model to study the inducible remodeling of heterochromatin architecture during differentiation and assess alternative strategies for removing H3K9/36me3 marks during nuclear reprogramming of different somatic and embryonic cell types. The work also generated the first rejuvenated tetracycline-inducible bovine somatic cell lines, which have been used to probe the pluripotent stem cell state in cattle.

9. Genetically engineered goats capable of producing female only offspring
(CONFIDENTIAL)

This project investigates a genetic system for the production of female only offspring that addresses existing animal wastage and associated animal welfare concerns in commercial animal production systems. The genetic system is based on a transgene that affects [redacted] the ability of sperm to successfully fertilise an egg following mating. The transgene only affects sperm that harbour a chromosome where the transgene is inserted. If inserted on the Y-chromosome, it will severely disadvantage fertilisation by Y-bearing sperm and the production of males. Hence, such a genetic system has the potential for female only offspring production. In our initial proof-of-concept study, the transgene was integrated on a single autosome. This enables to determine the impact of the transgene on transgene-carrying sperm by analysing the skewing of the normal 50/50 ratio of non-transgenic/transgenic offspring in favour of non-transgenic offspring. Transgenic male goats were generated and validated for correct testis-specific expression. Sperm from the transgenic goats was analysed by computer-assisted sperm analysis. Motion patterns were shown to be affected in a similar fashion compared to mouse sperm [redacted] This demonstrated that the genetic system was functional in the goats. However, analysis of embryos generated following artificial insemination of female goats showed a normal 50/50 ratio of non-transgenic/transgenic embryos. [redacted] With the understanding that the genetic system functions in principle in goats, we have now integrated [redacted] into a Y-chromosome location. The site-specific integration was undertaken in male goat somatic cells.

Following the confirmation of correct site-specific transgene insertion, we are now planning to generate animals from these cells. [redacted]

10. Adapting dairy cattle to hotter climates

The predicted increase in hot weather events due to a rapidly changing climate highlights the need to urgently adapt dairy cattle to these new conditions to lessen the associated increasing animal welfare burden, minimise lost productivity and achieve sustainability goals. We have identified two naturally occurring sequence variants in cattle that are associated with increased thermotolerance. One leads to a lighter coat colour, decreasing radiative heat gain and the other to a slick coat with shorter hair that is known from tropical cattle breeds and is associated with heat tolerance. Using genetic technologies, we are able to functionally evaluate these beneficial variants by directly introducing them into the genome, essentially within a single generation. We have introduced the sequence variant, that leads to an almost white coat colour into bovine cells derived from normally black and white Holstein Friesian dairy cattle. Following confirmation of the intended genotype, we have generated two calves which displayed a lightening of the normally black markings to a grey/silver colour. This for the first time validated the causative nature of a sequence variant for coat colour. In addition, we have extensively evaluated the genotype of the calves and the cell clones and cell line used in their production for any technology-induced off-target mutations and changes in the level of de novo mutations. Using an unbiased, sensitive approach (60X whole genome sequencing), we confirmed the full conversion to the intended genotype of the introduced sequence variant but have not found any evidence for the presence of unintended off-target mutations. Naturally, the genome will gain spontaneous de novo mutations in each generation. De novo mutations were detectable in our calves but were at the same level that is expected to be found in cattle. Two manuscripts reporting on the generation and phenotype of the lighter coat colour calves and the detailed genotype analysis are in the final stages of preparation.
As a next step, the intension is to optimise the introduction of the above-mentioned sequence variants not into cells but directly into bovine embryos. We have already generated embryos for one of the sequence variants which will soon be transferred for development to term. Ultimately, we are aiming to develop procedures that will enable us to generate cattle, modified with several beneficial variants already existing in cattle and improve the sustainability and welfare of dairy farming systems

b) any adverse effects of the organisms that have occurred, including any effects which relate to the matters described in section 6(d) and the principles of the Treaty of Waitangi (Te Tiriti o Waitangi)

In their decision for approval of ERMA application 200223, the Committee considered the potential for any adverse effects of the organisms to the environment and the health and safety of people as negligible. This was based on i) a track record of prior 10 year experience of safely containing GM livestock, ii) activities limited to livestock species that are easy to identify and contain, iii) a secure containment facility preventing escape into the environment, iv) disposal of all animals and products within the containment facility via a methodology previously agreed with Ngati Wairere, v) animals and their products not entering the human food chain, vi) restricted access to the containment facility that limits the number of people getting in close contact to the animals and vii) a mandatory set of controls to ensure potential risks are appropriately managed. Over the last ten years, the expected ability of the containment system to safely contain the GM animals was fully validated, with no animals attempting to or escaping containment. With no containment breach and farming according to resource management obligations, effects from the GM animals on the environment were limited to effects caused by farming livestock and the same as generated from conventional farming activities. Tissues and products of the animals were only used for scientific analyses and did not enter the human food chain. Developing and maintaining the GM animals in containment has also not resulted in any health and safety issues over and above risks associated with farming conventional livestock. To the contrary, the GM animals are kept under an

increased health standard reducing the risk for potential zoonotic infections. Taken together, this provides strong evidence that the GM animals have not and are highly unlikely to adversely affect the health and safety of people and communities. The GM animal activities are centred around research and undertaken in strict containment. Therefore, they cannot interfere with commercial activities and adversely affect the New Zealand economy. By contrast, the scientific knowledge gained has the potential to generate new commercial opportunities and associated economic benefits.
Moreover, it demonstrates that the controls are appropriate and an effective tool to manage the residual risk posed by the GM animal activities in outdoor containment.

Research involving experimental animals needs the approval by the responsible Animal Ethics Committee under the Animal Welfare Act. All our animal work was assessed and approved by the Animal Ethics Committee responsible for activities at our Ruakura facility.

Our research activities do not involve genetic material from native flora and fauna. To further be able to take into account the special relationship of Māori with their ancestral lands, water, sacred sites and respect principles of the treaty of Waitangi, a national Māori consultation had previously been undertaken. We had also an established Māori liaison process, which in the later stages had managed to merge into one group for the earlier GMF98009 and GMD02028 approvals, comprised of Ngati Wairere and Waikato Tainui representatives to have conversations about possible implications of the research with their special cultural relationships and the principles of the Treaty of Waitangi. After several initial meetings the contact was lost with their designated hapu representative when her mandate was withdrawn by the hapu. Subsequently, in an attempt to re-establish regular dialogue, we undertook a number of initiatives including:

- Inviting Ngati Wairere kaumatua and kuia to the Fieldays AgResearch dinner - which was successful at the time
- Seeking their assistance for various blessing ceremonies and powhiri held at AgResearch
- An approach was made to a senior Waikato Tainui leader with connections to Ngati Wairere who agreed to facilitate our relationship, ending sadly with his premature passing.
- An approach was made for further advice from staff of the Waikato Tainui Waikato Raupatu Trust with connections to Ngati Wairere.

Through contacts established during this period we discussed the possibility of holding a wananga at Hukanui for AgResearch staff hosted by Ngati Wairere and had requested attendance at the monthly marae meeting. Unfortunately though, we were unable to confirm a regular mana whenua representative for the containment centre.

More recently we have approached Te Haa o te whenua o Kirikiriroa Trust. Te Haa o te whenua o Kirikiriroa is an iwi group representing local mana whenua (Māori with historic ties to the Hamilton/Kirikiriroa area). This resulted in kaumatua and kuia representatives of Ngati Wairere offering to participate in the powhiri for the new AgResearch CE, Dr Sue Bidrose.

Moreover, our continuous efforts in communications with local Māori representatives have initiated consultation meetings with local Iwi/Māori partners within the *Waikato rohe*. In November 2019, a hui was held at Ruakura with Waikato Tainui representatives to discuss the different views regarding the use of contemporary genetic technologies for livestock applications, included ongoing and planned projects under ERMA 200223. Another consultation group hui was planned for March 2020 but had to be postponed due to COVID-19. The intension is to work towards forming a standing committee to regularly consult on our gene technology and build a long-term relationship for constructive engagement. We will seek advice going forward on how representation can be achieved.
We continue to work with our established contacts and Māori agri-business partners to ensure that the direction of the research is tika and consistent with Māori values, e.g, manaakitanga and mauri. Māori will be informed and involved in the research, including any trials, ethical requirements and opportunities

to set future direction. We discuss concerns and gather feedback from Māori involved in the primary industries sector, furthering reciprocal understanding how genome editing fits into Māori value-based farming systems. In addition, we participated in several huis with community members, the Te Nohonga Kaitiaki Research Team, the Co-innovation Interface Research Team (led by Maui Hudson, University of Waikato), and members of the Royal Society Te Aparangi gene editing panel and Māori Reference Group. These open discussion forums provide updates for the various GE projects and their diverse applications, with the long-term goal of developing a relationship for constructive consultation with *tangata whenua*. We also provided expert advice and primary industry case studies to the Royal Society Te Apārangi project on 'Gene Editing in Aotearoa' (RSNZ. Gene editing for the primary industries. https://royalsociety.org.nz/what-we-do/our-expert-advice/all-expert-advice-papers/gene-editing-for-the-primary-industries/.). As with the rest of the NZ society, Māori views and attitudes on genetic modification can be highly diverse. We therefore see it as important to also engage more widely and communicate risks and benefits offered by applications of rapidly advancing genetic technologies to reduce risk that it evades policy resolution, jeopardising its use for primary production and missing out on societal, sustainability and economic benefits. We reach out to a wide range of different sectors of the public through public lectures and engaging with teachers, students, and many diverse interest groups. As part of our research plan, public acceptance will be evaluated to provide a better understanding of how the emergence of more precise and save technologies changes public attitudes towards genetic modification.

In a more general context, AgResearch is engaged in fostering dialogue with Māori on impacts of new scientific knowledge and technologies. AgResearch along with other crown research institutes are evolving to understand and acknowledge the significant roles our Māori partners hold as kaitiaki, mana whenua, and Māori businesses along with their unique holistic view of balancing environmental, cultural, social and economic outcomes for their communities. In this context AgResearch has adapted our Māori engagement approach on how we share science opportunities, research findings and new tools and technologies as they relate to land use, agriculture and food.

In the earlier stages AgResearch's engagement with Māori could be viewed as transactional and related to specific projects meeting a specific need of a Māori entity or a wider sector need that happened to include a Māori entity as a participant e.g. an east coast hill country project focussed on establishing legumes included Māori Agribusiness Tangihanga Station of Wi Pere Trust. The specific need of Tangihanga Station focussed on weed control strategies to enable legume persistence. Part of the strategy including introduction of a biocontrol agent for thistle control and development of a decision tool for variegated thistle.

More recently AgResearch's relationship with key Māori partners is transforming our Māori engagement towards codeveloping research where there is mutual benefit throughout and beyond the partnership. Examples include:

- Exploring Future Platform and Rural Futures Multi-Agent Simulation Tool – a multi-disciplinary model of farmer behaviour and land-use change codeveloped with Aohanga Incorporation
- Social Return on Investment Tool – an investment tool that demonstrates the financial, social, cultural or environmental impact of land use investment codeveloped with Aohanga Incorporation
- Kaitiaki Land-use Decision Making Framework - brings ecosystems services and Matauranga Māori together for integrated farm system design codeveloped with Nga uri o te ngahere and utilised with Ngati Pahauwera Development Trust.
- AgINFORM – a next generation farm system model under development that enables land use optimisation within ecological boundaries and cultural values codeveloped with Nga uri o te ngahere, Ngati Pahauwera Development Trust, Onenui Station, Paroa Trust, Te Tumu Paeroa.
- Whaowhia te kete mātauranga (Fill your basket of knowledge) – a tool to explore the cultural provenance and value-add of Māori agribusiness value chains codeveloped with business units within Ngāti Porou Group Holding Ltd, Ngāti Porou Seafoods Ltd, Ngāti Porou Miere, Pakihiroa Farms Ltd and Ngāti Porou Forestry.

The short- and medium-term research needs of our Māori partners are heavily focussed on decision making tools related to land use, value chains and understanding the wider impacts and benefits i.e. social, cultural, environmental and economic. In time we expect these needs to evolve into other research areas, tools and technologies.

Taking advantage of developing and strengthening Māori relationships in the primary sector, we will integrate our engagement on genetic technologies to expand our dialogue and maximise constructive engagement with Māori on the potential impact of new scientific discoveries.

c) any beneficial effects of the organisms that have occurred in the first ten years, or that are forecast to occur over the next ten years

In the decision on ERMA 200223, the potential and the probability of the overall risks posed by the research programme have been assessed as negligible. The past ten years adds to a 20-year track record that fully validates this assessment. By contrast, the research programme has delivered on its promise on the development and increase of scientific knowledge. Further details on scientific breakthroughs are provided below, followed by complete documentation of research results documented in lists of scientific publications and conference presentations.

1. Addition of casein genes improve milk composition

The composition of milk has been optimized through the evolution of mammals to provide an optimal balance of protein, lipids and carbohydrate to support the growth and wellbeing of the newborn offspring. The most abundant proteins in milk, the caseins, are packaged into an ordered micelle, which facilitates an extremely high protein concentration. Similarly, the triacylglycerides in milk are packaged into a phospholipid membrane-bound globule, and many of the minerals and other micronutrients in milk are complexed to carrier proteins. The microstructures and complexes within milk are important for its dairy processing characteristics and presumably also its biological functions. Uniquely among mammals, humans consume milk from other species, as a major food source. However, cows' milk has a distinct protein repertoire compared with human milk, and the protein content of human milk is lower, and the lactose content is higher compared with cows' milk. Differences in viscosity and micelle characteristics also exist and micelle size has been shown to affect the processing properties of milk. Thus, there is the potential to alter the composition of cows' milk so as to better optimize its biological functionality as well as its processing characteristics as a food for the human population. The introduction of additional β - and κ -casein genes into the bovine genome has demonstrated the feasibility of altering milk characteristics through a transgenic approach. The high casein milk produced by the transgenic cows has distinctly altered characteristics that could provide improved dairy processing characteristics such as greater thermal stability and increased cheese yield due to a reduced size of the casein micelles. Increased levels in micronutrients and sialic acid, are likely to provide significant health benefit and additional value to dairy products.

2. Vaccine for MS based on rhMBP produced in transgenic cows

MS is an autoimmune neurodegenerative disease characterized by inflammatory lesions and demyelination in the central nervous system (CNS). Patients with this disease suffer from several disabilities like memory dysfunction, cognitive deficit and movement disorders. Approved drugs for treatment of MS that non-specifically inhibit the immune system offer no cure and are often associated with serious side effects. Human myelin basic protein (hMBP) is considered as the autoantigen in the MS pathogenesis process. Presence of hMBP in the cerebrospinal fluid at levels higher than normal (> 4 ng/mL) is a marker of active inflammation and myelin breakdown. Recent studies revealed that administration of the hMBP or its antigenic fragments to MS patients, reduced the severity of the disease through a tolerance mechanism. However, hMBP can only be isolated from human CNS tissues and has therefore a very limited availability. Due to the complex pattern of posttranslational modifications (PTM)

of hMBP, simple recombinant systems were incapable of producing biosimilar protein with equivalent properties to the human counterpart, but can be achieved with a cattle production system. Formulated into nanoparticles, rhMBP demonstrated efficacy as a therapeutic vaccine in an MS mouse model where it ameliorated MS symptoms in the disease model (Al-Ghobashy et al., 2017). This demonstrated the potential for the development of new efficacious therapeutic MS treatment options with the ability to produce rhMBP in large amounts in transgenic cattle.

3. Goats producing a highly efficacious therapeutic anti-cancer antibody

Therapeutic monoclonal antibodies (mAbs) represent one of the most important classes of pharmaceutical proteins to treat human diseases. Most are produced in cultured mammalian cells which is expensive, limiting their availability. Goats, striking a good balance between a relatively short generation time and copious milk yield, present an alternative platform for the cost-effective, flexible, large-scale production of therapeutic mAbs. Such a platform is especially well suited for NZ because it can leverage its competitive advantage in the primary sector, including disease-free status of livestock and world-leading research capabilities. We generated transgenic goats that produce a mAb in their milk that is commercially produced under the brand name Erbitux and approved for anti-cancer treatments. The transgenic goats produced an improved mAb version with the potential for enhanced effectiveness and better safety profile compared to treatments with Erbitux (Laible et al., 2020).
Following successful development and adoption, major economic benefits can be expected from mAb sales and treatment cost-saving to the NZ health sector. Importantly, the greater affordability of therapeutic mAbs produced in NZ goats can make treatment options more widely available and reduce disease-associated social costs. Success with this first goat-produced mAb will also generate new opportunities for the facilitated development of additional therapeutic mAbs associated with substantial future benefits.

4. Successful adaptation of RNA interference (RNAi) in livestock to control endogenous gene activities

RNAi technology allows to knockdown the activity of specific endogenous genes by simply overexpressing an interfering RNA that specifically targets the mRNA of an endogenous gene. The technology was shown in mice to be capable of producing phenotypes similar to disrupting a gene by modifying the DNA sequence of the gene itself. However, the few studies that have applied RNAi to modify important traits in livestock animals, were largely unsuccessful. We were able to develop an RNAi system that essentially completely disrupted the target gene activity in cattle, validating RNAi technology as a highly effective strategy to improve livestock traits in a direct and targeted way (Jabed et al., 2012). This bovine model now provides a unique resource to elucidate the biological functionality of an interfering RNA in milk.

5. Cattle producing hypoallergenic milk

Milk from dairy cows contains the protein BLG, which is not present in human milk. Consequently, BLG constitutes a major allergen in cows' milk with about 3% of babies and young infants affected by bovine milk allergies that can cause symptoms ranging from mild to live threatening. Removal of BLG from cows' milk reduces its allergenic potential and produces a milk that could provide a valuable source of nutrition for those children and adults that currently cannot consume milk due to allergic reactions that are directed against BLG. A variety of processing technologies, including enzymatic hydrolysis, can be used to reduce the allergenicity of milk proteins but they are expensive and may lead to the accessibility of additional allergy-causing peptides. Genetic modification offers an elegant strategy for the elimination of allergy causing proteins or peptides. Using a precise genetic approach, we have disrupted the BLG gene at the start of the encoded information, completely eliminating production of the allergenic protein (Wei et al., 2018). These cattle, modified for the ability to produce BLG-free milk, safely produce hypoallergenic cows' milk and make it accessible for sufferers of BLG allergies otherwise excluded from the high nutritional benefits of milk.

6. Germline complementation in sheep - new breeding approaches

Successful germline complementation, as demonstrated in our work to functionally evaluate the *NANOS2* gene, will provide a superior alternative to AI in extensive sheep farming systems, and deliver a scalable platform technology for rapidly disseminating diverse beneficial traits into extensively farmed livestock. By developing 'absolute transmitters', a new class of rams with a proven elite germline, we will short-cut years of conventional breeding efforts. This new 'natural AI' breeding scheme integrates with embryo-based genomic selection, which we have originally pioneered in dairy cattle. We have extended the research concept from sheep into dairy cattle, where embryonic complementation can more efficiently multiply elite embryo genotypes into absolute transmitters. The technology can further be adopted and commercialised by the beef industry and we have initiated the appropriate industry linkages through NZ-based stakeholders. For both sheep and cattle, absolute transmitters can long-term replace somatic clones for faster genetic gain, improving animal welfare outcomes. The programme can be flexibly tailored to different breeding objectives, environments and market demands to secure animal-based food production.

7. Kidney complementation in immune-compatible sheep – xenotransplantation

Sheep physiology and anatomy is similar to humans, making them potential donors to meet the global demand for transplantable organs. They are also culturally more widely accepted than pigs, providing a possible alternative to this species. However, a critical barrier to the use of sheep organs for xenotransplantation is immune rejection due to xenoantigens. To address this problem and better match sheep organs to human recipients, we have genetically removed two known xenoreactive antigens from sheep. These animals can be bred to generate a flock of immune-compatible sheep for pre-clinical testing. New technologies are also moving from using livestock organs to using these species as hosts for growth of human organs. This concept proposes disrupting a master regulatory gene to prevent organ development in a livestock host, followed by complementation with human cells that populate the empty niche and generate the organ. We have begun to validate this concept in sheep by studying the effect of knocking out *SALL1* and rescuing the phenotype through embryo complementation with heterologous, wild-type sheep cells. These are first steps towards ultimately generating a sheep-human transplantation system in the future. Our focus is on the kidney, which comprises 66% of all transplants and >80% of patients on the waiting list. Harvesting of other immune-compatible biomaterials, including decellularized, non-living tissues, is also possible. Such products can be produced and marketed in NZ (scenario 1), produced in NZ but marketed overseas (scenario 2) or produced and marketed overseas (scenario 3). This is because the logistical pathways for preserving and transporting transplantable products are already well-established and continue to be refined. In the current regulatory and public environment, precise genetic modification for therapeutic purposes is permissive, i.e. Medsafe NZ-approved trials with GM products are already underway in NZ.

8. Rapid genetic improvement of cattle by embryo-mediated introduction of precise, gene-specific sequence modifications

Present precision genetic modification strategies rely largely on the introduction of the modification into the genome of somatic cells and the subsequent generation of live animals by SCT. However, even 20 years after Dolly, SCT is still hampered with poor efficiencies for the production of live offspring and losses after birth are also considerably higher in SCT clones compared with sexually derived animals. As an alternative to SCT, zygote injections of genome editors have been successfully used to precisely change the genome of zebrafish, mice, rats, and rabbits. In these fast-reproducing species with large numbers of offspring, non-mosaic homozygosity of the desired modification can be quickly and cost-efficiently achieved by breeding from animals that are heterozygous and/or mosaic carriers of the modification. In livestock animals with longer generation intervals and typically smaller litter sizes, especially in ruminant species, SCT has been thus far the preferred approach. We developed a new method combining zygote injections in bovine with biopsy pre-implantation diagnosis and embryo cryopreservation, prior to transfer of fully validated embryos in recipients for development to term. This proved the ability of zygote-mediated genome editing to generate precision-edited cattle for the

dissemination of valuable phenotypes and provides a new avenue for the rapid genetic improvement of livestock. This also includes the use of epigenome editors, based on histone demethylase KDM4, a chromatin-modifying enzyme that removes post-translational trimethylation from transcriptionally silent heterochromatin. This epigenetic modulator opens up chromatin, increasing its accessibility at difficult target loci to enable multi-gene editing, simultaneously enhancing cloning efficiency.

9. Genetically engineered goats capable of producing female only offspring
   (CONFIDENTIAL)

Development of a technology for gender-selected offspring as a superior naturally sexed semen product in dairy farming will result in improved animal welfare, higher productivity and reduced environmental footprint. [redacted] Successful outputs will deliver a commercially viable technology that achieves >90% female offspring that have not been genetically modified, born from natural, non-genetically modified dairy cows. The findings generated through this programme will deliver a validated patented new technology to produce a breakthrough commercial-ready superior sexed semen product to transform dairy farming in New Zealand to: 1) improve animal health and welfare through elimination of reduced fertility rates suffered with existing sex-sorted semen products and providing a tool to increase the value of unwanted bobby calves 2) increase productivity and herd breeding worth (BW) by enabling farmers to select their top producing cows for generating replacement heifers to drive genetic gain across the herd and breeding for quality dairy-beef cross male calves with a portion of the rest of the herd. Higher BW herds increase productivity and enable lower stocking rates resulting in reduced leaching and emissions and 3) increase biosecurity safety and traceability by generating more closed herd high-BW replacements [redacted] Communication and education of the technology and its significant advantages to stakeholders will be imperative to assure regulatory, industry and consumer acceptance. [redacted]

10. Proof for the causative nature of a natural sequence variant for lightening coat colour in cattle

High-producing Holstein Friesian dairy cattle have a characteristic black and white coat pattern where black frequently comprises a large proportion of the coat. Compared to a light coat colour, black absorbs more solar radiation translating into radiative heat gain which is a contributing factor to heat stress in cattle, negatively impacting on their production levels, fertility and welfare. To better adapt Holstein Friesian dairy cattle to the rapidly changing climatic conditions with predictions for more frequent and prolonged hot temperature patterns, we introduced a specific mutation proposed as the causative variant responsible for the semi-dominant colour dilution phenotype seen in Galloway and Highland cattle. The modified calves displayed a novel pattern of grey and white markings and absence of any black areas. This, for the first time, verified the causative nature of this sequence variant for diluting the black coat colour in cattle. With these modified animals, it is now possible to dissect the effects of the introgressed sequence variation and other interfering allelic variants that might exist in individual cattle and accurately determine the impact of the sequence change on important health, welfare and production traits. This will improve the accuracy of predicting phenotypic performance from genomic sequence information, accelerating genetic gain. In addition, it proved targeted genetic improvement as a promising novel breeding approach for the rapid adaptation of livestock to changing climatic conditions. Although we have demonstrated it for a dairy breed, the strategy could be readily applied to beef breeds such as Black Angus. Projected onto a global scale, even modest improvements of eco-productivity from colour-diluted cattle would translate into substantial environmental benefits.

11. Availability of extensive safety data in support of science-based risk assessment

The research activities including the development and characterisation of genetically modified cattle, goat and sheep generated a wealth of scientific information. Such data are relevant for the assessment of the biosafety risks from such activities. Availability of such data is a prerequisite for science-based assessment and will support regulators and policy makers in their decision process and accurately inform commercial stakeholders and consumers to facilitate appropriate commercialisation of modified animals and their products in the future.

12. Communication of scientific progress to the wider community

We have been communicating our scientific findings, technological progress and future developments with stakeholders, regulators, politicians, teachers, students, journalists and the wider public. This includes numerous invited presentations at national and international conferences (see 'Refereed Conference Proceedings' and 'Invited Conference Presentations' below); advising the Royal Society of New Zealand (RSNZ) campaign on genome editing by providing primary industry case studies; extended engagement with industry groups, the public and Māori stakeholders to inform the debate on genome editing, for example, through industry meetings (e.g. Fieldays); public outreach, for example, via the Maurice Wilkins Centre-led outreach initiative (e.g. Biology Teacher Professional Deveklopment Days and school visits); community engagement, for example, via the RSNZ Public lecture series: Genome Methods for NZ and contributions to the Science learning hub ("Transgenic cows – introduction. www.sciencelearn.org.nz/resources/834-transgenic-cows-introduction")

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B Brophy, S Cole, J Wei, DN Wells, G Laible (2018). Modification of Bovine Coat Colour Using CRISPR-Cas9 Homology Directed Repair (Aug 30-31, QRW Queenstown, New Zealand)

SJ Appleby, AJ Davidson, B Oback (2018) Generating immune-compatible sheep for xenotransplantation (Aug 30-31, QRW Queenstown, New Zealand)

B Forrester-Gauntlett, L Peters, B Oback (2018) Grainyhead-like 2 is required for inner ear-like organoid formation from mouse embryonic stem cells (Aug 30-31, QRW Queenstown, New Zealand)

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B Oback, B Huang, V Verma and D Harris (2013) The exceptional epiblast: pluripotency signalling in non-murine species (Jan 19–22, DABE Forum "Natural and Induced Pluripotency in Domestic Species" (Proceedings of the 39th Annual Conference of the IETS Society, Hannover, Germany)

DN Wells, B Oback, G Laible (2012) Cloning livestock: a return to pluripotent stem cells (Sept 2-6, 'Adapting to a Changing World' The XII ABIC International Conference on Agricultural Biotechnology, Rotorua, New Zealand)

G Laible (2011) Cell-mediated transgenesis of livestock for biopharming and improved dairy products. (The 2011 International Conference for Bioeconomy, Tianjin, China).

B Huang, T Li, L Alonso-Gonzalez, R Gorre, S Keatley, A Green, P Turner, P Kumar, V Verma, B Oback (2011)Expression of a non-viral poly-promoter vector induces pluripotency in quiescent bovine cells under chemically defined conditions (June 15-18, International Society for Stem Cell Research (ISSCR) 9th Annual Meeting, Toronto, Canada)

A Jabed, R Broadhurst, S Wagner, R Martinus, G Laible (2010) Demonstration of RNAi-mediated knockdown of a major ruminant specific milk allergen in a transgenic mouse model. (14-19 January, Keystone Symposia for Molecular and Cellular Biology - RNA Silencing: Mechanism, Biology and Application (A7), Keystone, CO, USA)

A Jabed, R Broadhurst, S Wagner, R Martinus, G Laible (2010). RNAi-mediated reduction of a major ruminant specific milk allergen in a transgenic mouse model. (13 May, Waikato Sustainable Bioeconomy Student Poster Conference, Hamilton, New Zealand)

A Jabed, R Broadhurst, S Wagner, R Martinus, G Laible (2010). RNAi-mediated reduction of a major ruminant specific milk allergen in a transgenic mouse model. NZBIO 2010 Conference (22-24 March, Auckland, New Zealand)

Invited scientific presentations

2020 ADPI Dairy Ingredients Symposium, Santa Barbara, USA
2019 Maurice Wilkins-GIBH Joint Symposium, Auckland, New Zealand
2019 29th Annual Queenstown Molecular Biology Meeting (QMB)
2019 3rd Asia-Pacific Kidney Development Workshop, Queenstown, New Zealand
2019 Transgenic Animal Research Conference XII, Tahoe City, USA
2019 American Dairy Science Association Annual Meeting, Cincinnati, USA
2019 Digital Biology Applications, Networking Forum & Masterclass, Auckland, New Zealand
2018 FASEB Science Research Conference, Florence, Italy
2018 Seminar, Institute of Farm Animal Genetics, Mariensee, Germany
2018 QMB Satellite Gene Technologies- Applied Genetic Technologies, Queenstown, New Zealand
2018 Annual Obex Embryology meeting (Fertility Associates), Auckland, New Zealand
2018 Special Symposium, Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany
2017 World Conference on Sheep, Beijing, China
2017 Xenotransplantation Symposium, NZEno, Auckland, NZ
2017 27th Annual Queenstown Research Week (QRW), New Zealand, "Reproduction" Satellite
2017 "How to make the most of CRISPR genome engineering technologies" workshop, University of Auckland, New Zealand
2016 FASEB Science Research Conference, Lisbon, Portugal
2016 Conference on Large Animal Genetic Engineering for Biomedical and Agricultural Applications, Bethesda, USA
2016 Dolly@20 symposium, Edinburgh, Scotland
2016 Workshop on Transgenic Livestock, Bangaluru, India
2016 26th Annual Queenstown Research Week (QRW), Nelson, New Zealand, "Stem Cells & Regenerative Medicine" Satellite
2015 Seminar, Institute of Farm Animal Genetics, Mariensee, Germany
2015 ABIC 2015, Melbourne, Australia
2015 18th ADNAT Convention, Hyderabad, India
2015 Seminar, National Institute of Animal Biotechnology, Hyderabad, India
2015 NZBIO, Wellington, New Zealand
2015 25th Annual Queenstown Research Week (QRW), New Zealand, "Animal Genomics" Satellite
2015 Annual ASAIHL (Association of Southeast Asian Institutions of Higher Learning) conference, Esfahan, Iran
2015 1st International and 9th National Biotechnology Congress, Tehran, Iran
2014 OECD Workshop Removing barriers to the uptake of GM animals as sustainable solution to food security & safety, Geelong, Australia
2014 New Technology Workshop, Auckland, New Zealand
2014 International Conference on New Ideas in Agriculture, Isfahan, Iran
2014 24th Annual Queenstown Research Week (QRW), New Zealand, Co-organiser Symposium "Development and Reproduction"
2014 Annual Otago Genomics Conference 'Translational Genetics', Dunedin, New Zealand
2013 Transgenic Animal Research Conference IX, Tahoe City, USA
2013 AGCARM Summer Conference, Auckland, New Zealand
2013 Annual Stem Cell Researchers Network, University of Auckland, New Zealand
2013 23rd Annual Queenstown Research Week (QRW), New Zealand, Symposium "Epigenetics – Spanning the Spectrum"
2013 Seminar, Max Planck Institute for Molecular Genetics, Berlin, Germany
2013 Seminar, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany
2013 Department Seminar, Maurice Wilkins Centre, New Zealand
2013 Department Seminar, University of Waikato, New Zealand
2013 Reproductive Science Symposium, Wellington, New Zealand

2013 39th Annual Meeting of the International Embryo Transfer Society (IETS), Hannover, Germany
2011 Transgenic Animal Research Conference VIII, Tahoe City, USA
2011 International Conference for Bioeconomy, Tianjin, China
2011 RDNZ Education - Enhancing your professional development), Taupo, New Zealand.
2011 Centre for Reproduction and Genomics (CRG), Invermay, New Zealand, 3rd Annual Research Colloquium

Hui

2019 Genetic Engineering Consultation Group Meeting, Ruakura, New Zealand
2019 Huihuinga Māori: Gene Editing in Aotearoa to draft a note that informs the Gene Editing Panel's Māori Reference Group, Hopuhopu, New Zealand
2018 Huihuinga Whānau Māori: Gene Editing in Aotearoa – Primary Industries, Wellington, New Zealand
2018 Huihuinga Whānau Māori: Gene Editing in Aotearoa – Primary Industries, Hamilton, New Zealand
2011 Māori Liaison Group meeting, Hamilton, New Zealand
2011 Ngā Kaihautū Tikanga Taiao, Hamilton, New Zealand
2011 Māori Liaison Group meeting, Hamilton, New Zealand
2010 Māori Liaison Group meeting, Hamilton, New Zealand

Community engagement

2020 Hamilton Girls' High School, Hamilton, New Zealand
2017 Biology Teacher Professional Development Day, Wellington, New Zealand
2017 Biology Teacher Professional Development Day, New Plymouth, New Zealand
2017 Royal Society of New Zealand, Public lecture series: Genome Methods for NZ, Hamilton, New Zealand
2015 Presentation to EPA and Ministry for the Environment delegation on 'Gene Editing and Stem Cells'.
2014 Australia and New Zealand Dairy Co-operative Leaders' Forum, Auckland, New Zealand
2013 Biology Teacher Professional Development Day, Napier, New Zealand
2013 Biology Teacher Professional Development Day, Christchurch, New Zealand

Research grants

The research was supported by multiple, fully contestable multi-year government grants from the Ministry of Science and Innovation and Ministry of Business, Innovation and Employment. Further financial support was awarded to some of the projects through fully contestable grants from Callaghan Innovation, the Royal Society of New Zealand, the Cure Huntington's Disease Initiative Foundation, and the Canadian Institutes of Health Research.

Media coverage (examples)

- Kiwi goat milk could play role in fighting cancer https://www.newshub.co.nz/home/rural/2020/06/kiwi-goat-milk-could-play-role-in-fighting-cancer.html
- Genetically modified goats can produce cancer drugs in their milk https://www.newscientist.com/article/2245887-genetically-modified-goats-can-produce-cancer-drugs-in-their-milk/
- How genetically modified New Zealand goats could help fight cancer https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=12339750?
- Genetically engineered goats could be the key to mass-producing cancer drugs https://www.digitaltrends.com/news/genetically-engineered-goats-cancer-drugs/
- The Guardian interview (20 December 2019) From red seaweed to climate-smart cows: New Zealand leads the fight against methane https://www.theguardian.com/world/2020/jan/01/from-red-seaweed-to-climate-smart-cows-new-zealand-leads-the-fight-against-methane
- Radio New Zealand interview - Nine to Noon (21 October 2019) https://www.rnz.co.nz/national/programmes/ninetonoon/audio/2018718592/calls-for-change-to-laws-on-gene-editing
- Gene-edited NZ pigs: organ donors of the future? (6 October, 2019) https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=12274061
- Scientists given $10m to breed 'smart' cattle using gene editing (2 October 2019) https://www.stuff.co.nz/business/farming/116270918/scientists-given-10m-to-breed-smart-cattle-using-gene-editing
- Cow of the future? How gene-editing could help dairy's climate problem (30 Sept 2019) https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=12272362
- Radio New Zealand interview - Morning Rural News (25 September 2019) https://www.rnz.co.nz/national/programmes/ruralnews/audio/2018714745/morning-rural-news-for-25-september-2019
- New-Zealand tentatively reopens debate into dairy gene editing (2018) https://www.dairyreporter.com/Article/2018/11/28/New-Zealand-tentatively-reopens-debate-into-dairy-gene-editing
- Kiwi scientists believe they can eliminate allergies (2018) https://www.newstalkzb.co.nz/on-air/mike-hosking-breakfast/audio/goetz-laible-kiwi-scientists-find-method-to-remove-milk-allergies/
- Foodevolution, organised public movie screening and panel Q&A (Queenstown 2018)
- GE cow's offspring show 'super-milk' potential (2017) https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=11785868
- Goat milk promises cheap cancer treatments: New Zealand scientists (2016) http://www.globaltimes.cn/content/972788.shtml
- Goat Milk Promises Cheap Cancer Treatments: New Zealand Scientists (2016) https://www.nzedge.com/news/goat-milk-promises-cheap-cancer-treatments-new-zealand-scientists/
- TV interview at Annual ASAIHL (Association of Southeast Asian Institutions of Higher Learning) conference on GE technologies in New Zealand, Esfahan, Iran (2015)

- The Guardian - GM cow designed to produce milk without an allergy-causing protein (2012) https://www.theguardian.com/science/2012/oct/01/gm-cow-milk-alllergy-protein
- CBS News - Scientists create cow that produces hypoallergenic milk (2012) https://www.cbsnews.com/news/scientists-create-cow-that-produces-hypoallergenic-milk/
- New Zealand Scientists Breed Cow to Make Low-Allergy Milk (2012) https://www.wsj.com/articles/SB10000872396390444004704578031333390178130
- ABC Science - GM cow produces allergy-free milk (2012) https://www.abc.net.au/science/articles/2012/10/02/3601916.htm
- Cow Engineered to Make Hypoallergenic Whey-Free Milk (2012) https://www.scientificamerican.com/article/cows-engineered-to-make/
- GM cows make 'low allergy' milk (2012) https://www.bbc.com/news/health-19785006
- The Dominion Post feature “Science still key to our future” covering our bovine iPS work (Sept 2011) http://www.stuff.co.nz/business/farming/5621787/Science-still-the-key-to-our-future

10-year Summary of Animal Numbers reported under On Farm Management

Summary to period ending 30/06/2020 (alignment with annual reporting)

Animal Numbers 28/04/2010 – 30/06/2020 (Births exclude still born or animals which die soon after birth reported in Animal Ethics Reports, Aged In and Out records changes in animal age)

Reporting period	Open	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing
Cattle									
Casein (ERMA200223)									
28/04 to 30/06/10	**69**								**69**
1/07/10 to 30/06/11	**69**				20	20	5		**64**
1/07/11 to 30/06/12	**64**	2			5	5	36		**30**
1/07/12 to 30/06/13	**30**						10		**20**
1/07/13 to 30/06/14	**20**	3			1	1	9		**14**
1/07/14 to 30/06/15	**14**	4					1		**17**
1/07/15 to 30/06/16	**17**				5	5	2		**15**
1/07/16 to 30/06/17	**15**	1			2	2	3		**13**
1/07/17 to 30/06/18	**13**	1			1	1	2		**12**
1/07/18 to 30/06/19	**12**						10		**2**
1/07/19 to 30/06/20	**2**						2		**0**
Total Casein	**0**	**11**	**0**	**0**	**34**	**34**	**80**	**0**	**0**
Total Casein held or developed			**80**						
MBP (ERMA200223)									
28/04 to 30/06/10	**7**								**7**
1/07/10 to 30/06/11	**7**	1			1	1	1		**7**
1/07/11 to 30/06/12	**7**	2			3	3	5		**4**
1/07/12 to 30/06/13	**4**	1			1	1	1		**4**
1/07/13 to 30/06/14	**4**	1			1	1	2	1	**2**
1/07/14 to 30/06/15	**2**				1	1	1		**1**
1/07/15 to 30/06/16	**1**				1	1			**1**
1/07/16 to 30/06/17	**1**	1					2		**0**
Total MPB	**0**	**6**	**0**	**0**	**8**	**8**	**12**	**1**	**0**
Total MPB held or developed			**13**						
rhLF (ERMA200223)									
28/04 to 30/06/10	**19**								**19**
1/07/10 to 30/06/11	**19**	10			16	16	4		**25**
1/07/11 to 30/06/12	**25**	8			7	7	12		**21**
1/07/12 to 30/06/13	**21**				4	4	11		**10**
1/07/13 to 30/06/14	**10**						10		**0**
Total rhLF	**0**	**18**	**0**	**0**	**27**	**27**	**37**	**0**	**0**
Total rhLF held or developed			**37**						
FSH - (ERMA200223)									
28/04 to 30/06/10	**2**								**2**
1/07/10 to 30/06/11	**2**						2		**0**
Total FSH	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
Total FSH held or developed			**2**						

Reporting period	Open	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing
BLg - (ERMA200223)									
1/07/11 to 30/06/12	**0**	1			1	1			**1**
1/07/12 to 30/06/13	**1**				1	1			**1**
1/07/13 to 30/06/14	**1**								**1**
1/07/14 to 30/06/15	**1**								**1**
1/07/15 to 30/06/16	**1**	17					1		**17**
1/07/16 to 30/06/17	**17**	10			21	21			**27**
1/07/17 to 30/06/18	**27**	13			21	21	20		**20**
1/07/18 to 30/06/19	**20**				8	8	1		**19**
1/07/19 to 30/06/20	**19**				3	3	3		**16**
Total BLg -	**16**	**41**	**0**	**0**	**55**	**55**	**25**	**0**	**16**
Total BLg- developed			**41**						
Erbitux (ERMA200223)									
1/07/10 to 30/06/11	**0**	1							**1**
1/07/11 to 30/06/12	**1**				1	1			**1**
1/07/12 to 30/06/13	**1**				1	1			**1**
1/07/13 to 30/06/14	**1**								**1**
1/07/14 to 30/06/15	**1**								**1**
1/07/15 to 30/06/16	**1**								**1**
1/07/16 to 30/06/17	**1**								**1**
1/07/17 to 30/06/18	**1**								**1**
1/07/18 to 30/06/19	**1**						1		**0**
Total Erbitux	**0**	**1**	**0**	**0**	**2**	**2**	**1**	**0**	**0**
Total Erbitux developed			**1**						
Coat Colour (ERMA200223)									
1/07/17 to 30/06/18	**0**	4						1	**3**
1/07/18 to 30/06/19	**3**				3	3	3		**0**
Total Coat Colour	**0**	**4**	**0**	**0**	**3**	**3**	**3**	**1**	**0**
Total Coat Colour developed			**4**						
KDM4B (ERMA200223)									
1/07/17 to 30/06/18	**0**	1							**1**
1/07/18 to 30/06/19	**1**				1	1			**1**
1/07/19 to 30/06/20	**1**				1	1			**1**
Total KDM4B	**1**	1	0	0	2	2	0	0	**1**
Total KDM4B developed			**1**						
Total Cattle developed or held under ERMA/EPA approvals (Tg and non Tg progeny) during period									**179**
Conventional Cattle									
28/04 to 30/06/10	**71**								**71**
1/07/10 to 30/06/11	**71**		8	2			11		**66**
1/07/11 to 30/06/12	**66**		9		1	1	22		**53**
1/07/12 to 30/06/13	**53**		34		7		23		**71**
1/07/13 to 30/06/14	**71**	30	112	110	1	1	7	2	**94**
1/07/14 to 30/06/15	**94**		84	29			29		**120**
1/07/15 to 30/06/16	**120**		32	24	94	94	13		**115**
1/07/16 to 30/06/17	**115**	8	11	64	48	48	7		**63**
1/07/17 to 30/06/18	**63**			12	5	5	10		**41**
1/07/18 to 30/06/19	**41**		64		5	5	5		**100**
1/07/19 to 30/06/20	**100**			5			9	1	**85**
Total Conventional	**85**	**38**	**354**	**246**	**161**	**154**	**136**	**3**	**85**
Total Conventional involved or on Facility			**463**						

Reporting period	Open	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing
Goats									
Erbitux & Enbrel (ERMA200223)									
1/07/10 to 30/06/11	**0**	4	21		23	23	16	2	**7**
1/07/11 to 30/06/12	**7**	10			13	13	1	1	**15**
1/07/12 to 30/06/13	**15**	25			32	32	4		**36**
1/07/13 to 30/06/14	**36**	8			39	39	10	1	**33**
1/07/14 to 30/06/15	**33**	12			28	28	3	1	**41**
1/07/15 to 30/06/16	**41**	3			11	11	1		**43**
1/07/16 to 30/06/17	**43**	33			30	30	28	2	**46**
1/07/17 to 30/06/18	**46**	9			26	26	16		**39**
1/07/18 to 30/06/19	**39**	4	4		23	23	14		**33**
1/07/19 to 30/06/20	**33**	1			7	7	7		**27**
Total Erbitux & Enbrel	**27**	**109**	**25**	**0**	**232**	**232**	**100**	**7**	**27**
Total Erbitux or Enbrel held or developed			**134**						
non Med inherit (ERMA200223)									
1/07/14 to 30/06/15	**0**	23			23	23			**23**
1/07/15 to 30/06/16	**23**						7	2	**14**
1/07/16 to 30/06/17	**14**						14		**0**
Total TCR	**14**	**23**	**0**	**0**	**23**	**23**	**21**	**2**	**0**
Total non Med inherit developed			**23**						
Total Goats developed or held under ERMA/EPA approvals (Tg and non Tg progeny) during period									**157**
Conventional Goats									
28/04 to 30/06/10	**0**								**0**
1/07/10 to 30/06/11	**0**		76		40	40	12	1	**63**
1/07/11 to 30/06/12	**63**	14			51	51	14	1	**62**
1/07/12 to 30/06/13	**62**	11			24	24	27	3	**43**
1/07/13 to 30/06/14	**43**	59			51	51	20	1	**81**
1/07/14 to 30/06/15	**81**	26			40	40	15	4	**88**
1/07/15 to 30/06/16	**88**		2		63	63	21	5	**64**
1/07/16 to 30/06/17	**64**	35			65	65	37	7	**55**
1/07/17 to 30/06/18	**55**				40	40	14		**41**
1/07/18 to 30/06/19	**41**			4	21	21	7		**30**
1/07/19 to 30/06/20	**30**						2		**28**
Total Conventional	**28**	**145**	**78**	**4**	**395**	**395**	**169**	**22**	**28**
Total Conventional involved			**223**						

Reporting period	Open	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing
Sheep									
AI on Hooves									
1/07/18 to 30/06/19	**0**	2			2	2			**2**
1/07/19 to 30/06/20	**2**	19			17	17	4	1	**16**
Total AI on Hooves	**16**	**21**	**0**	**0**	**19**	**19**	**4**	**1**	**16**
Total AI on Hooves developed			**21**						
Total Sheep developed under ERMA/EPA approvals (Tg and non Tg progeny) during period									**21**
Conventional Sheep									
1/07/16 to 30/06/17	**0**		62						**62**
1/07/17 to 30/06/18	**62**				52	52	7		**55**
1/07/18 to 30/06/19	**55**		119	1	42	42	14		**159**
1/07/19 to 30/06/20	**159**	12		3	71	71	66	7	**95**
Total Conventional	**95**	**12**	**181**	**4**	**165**	**165**	**87**	**7**	**95**
Total Conventional involved			**193**						

The preceding tables provide a summation of animal numbers by species over the 10 year reporting period in the various development lines that are linked to the EPA approval. This includes transgenic and non-transgenic animals (progeny) and the conventional animals (Cattle) which are used to support the programmes or allowed on to the Facility with MPI approval for pasture control purposes.

Where opening numbers are present for Transgenic lines these are animals which were previously field tested or developed under the GMF98009 or GMD02028 cattle approvals and transferred to the ERMA200223 approval following approval of the application. The 28th of April 2010 was recorded as the official transfer date between approvals as these cattle were already on the Animal Containment Facility.

Goats developed under the GMD09016 approval physically transferred from indoor facilities to the Animal Containment Facility when practical after approval of ERMA200223 during 2010.

Of the total 463 conventional cattle over the 10 yr period, 274 of these have been on the facility for pasture control purposes only or were unsuitable for use in the cattle program.

All animals identified as ‘Killed’ or ‘Deaths’ in the preceding tables have been disposed of in offal holes within the Animal Containment facility.

For management purposes, as previously identified, the facility is treated as a separate small farm within the main Ruakura Farm. It is fully self-contained apart for some machinery requirements and specialist staffing.

Animals on the facility have been managed in a way which is the normal farming practice in New Zealand, grazing outdoors on pasture, with some crops, and the supplementary feeding of hay, balage or concentrates when required.

This consists of daily shifts and restricted intakes depending on the age of the animal and its feed requirements. Examples are stage of pregnancy, lactating or rearing calf or kid, empty, young growing animals, etc.

All animals are regularly monitored for live weight and health status and goats can at times receive a higher proportion of their daily intake as supplementary feed, as concentrates, to reduce their impact on pasture availability for cattle and often have access to covered shelter in inclement weather.

Surplus pasture is conserved when possible for use in periods of low growth, as balage, silage or hay and if required purchasing of extra supplement (meal) which enables maintenance of an adequate annual feed supply.

Regular pasture renewal is carried out with at least 10% of the facility receiving some form of renovation annually. Mineral supplementation has been carried out using a mineral dispensing system through the water troughs or via direct animal supplementation for assisting Facial Eczema control and other normal mineral deficiencies during identified periods of risk, as occurs on many farms.

Maintenance fertiliser has been regularly applied over the period to maintain soil and pasture health and selective additional Nitrogen (Urea) at low rates, has also been used at times to boost pasture growth rates if needed.

Cattle over 2 yrs of age have been TB (Bovine Tuberculosis) tested 3 times in the 10 year period as required by National TB Eradication programme, with negative results each time.

In June 2018 the Ministry for Primary Industries Mycoplasma Bovis eradication program issued the Ruakura Farm with a 'Notice of direction' for animal tracing purposes in June 2018 which included the Animal Containment Facility because of its location within the Ruakura Farm. Biosecurity measures were revised and increased relative to both Facility and Farm entry. The 'Notice of direction' was revoked in August 2018 following clear test results.

Covid 19 Level 4 and 3 restrictions only impacted staff or science activities, animal care and welfare requirements continued as normal, with supplementary feeding as dry conditions impacted grass growth during this period.

Milk Production

Milk production from cows continued in the first 4yrs of the approval with milk that wasn't used to feed calves or needed for science analysis, stored and then irrigated to pasture following treatment via fermentation as per our previous approvals as permitted via resource consent.

In the following year the milk from the cows which calved was all fed to their calves and in subsequent years no GM cows have calved specifically for seasonal milk production. Those that did calve, reared their own progeny and small sample volumes were collected for science analysis only over a short period.

The milk from the GM goats which kidded and were milked during the period was either used to feed kids, for science analysis or frozen on the facility.

This has meant for the last 5 years there was no milk stored for surplus disposal by irrigation to pasture.

Ruakura Animal Ethics Committee (RAEC)

All activities were under the oversight of RAEC and undertaken with the appropriate AE approvals according to the Animal Welfare Act 1999.
All AE approvals for the experimental animal work were for the durations of a period of approximately one year. Upon expiry of an AE approval, a new approval was sought to continue the animal work under a new approval.
Activities and the health status of animals were regularly reported to RAEC in the form of quarterly interim reports.
Animal treatments and care was overseen by a registered veterinarian and animal welfare officer directly reporting to and participating in RAEC meetings.

MPI Verification Services Auditing

MAF continued the approval of the Animal Containment Facility (ACF) for use under the ERMA200223 approval and has verified compliance with the MAF/ERMA New Zealand Standard: 154.03.06 'Containment Standard for the Field Testing of Farm Animals' and the specific controls of the ERMA 200223 approval.
Aspects of the development work are also covered by MAF/ERMA New Zealand Standard:154.03.03 'Containment Facilities for Vertebrate Laboratory Animals' and MAF/ERMA New Zealand Standard: 154.03.02 'Facilities for Microorganisms and Cell Cultures: 2007a'.

As part of the decision for ERMA200223 the frequency of supervisory auditing by MAF was reduced from 3 monthly to 6 monthly.

During the period Governmental changes in 2011/12 have seen MAF become MPI and at a similar time ERMA became the EPA.

During 2016 AgResearch Ltd changed the overall structure of the many Containment Facilities it operated to have one Operator for the corporate entity.
At Ruakura this enabled the combining of four separate containment facilities into one Ruakura site transitional and containment facility with identified areas operating to specific standards with designated operators. This has meant a change to how MPI audit results for the ACF have been presented since then.

In summary there has been no breach of containment and no restrictions on the ACF's ability to operate imposed by MPI during the 10-year period.
In the 10 years, we have had 2 Non-Compliances (minor) for internal auditing reasons and 1 (also minor) related to incomplete register records.

Again, this provides strong evidence that the controls and MPI oversight are appropriate to manage the residual risk posed by the GM animal activities in outdoor containment.