Annual Report to Environmental Protection Authority for Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending 30th June 2015

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Operator / Manager
AgResearch Ruakura
Animal Containment Facility
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</tbody>
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**Summary of Activities for the period 1st July 2014 to 30th June 2015**

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

**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, which were field tested or developed under the GMF98009 or GMD02028 approvals continue to be maintained under the ERMA200223 approval and all new cattle development activities are now only being undertaken under the ERMA200223 approval.

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

Cattle and Goat activities, other than the maintenance or growing of animals, have been the calving and milking of cows, flushing eggs from fertile animals, kidding of recipient goats, milking of transgenic animals and the transfer of embryos to recipient animals.

These transferred embryos 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 have been undertaken with the approval of the Ruakura Animal Ethics Committee and all activity approvals have been renewed during the year.

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

**Unforeseen adverse effects resulting from the genetic modifications**

There have been no unforeseen adverse effects identified during this period.

**Iwi liaison group relationship development and management activities**

The ERMA200223 Liaison Group has 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, despite numerous attempts, no progress has been made in resolving this directly to date.

Informal contact with one original monitoring group member and Tainui Group Holdings continues to provide some updating on activities at Ruakura.

AgResearch’s Portfolio Leader – Māori Agribusiness who has local affiliations, has been working to build a relationship with Ngati Wairere for Liaison Group and other Ruakura initiatives of interest to Ngati Wairere and Tainui. Progress has been slow, but positive and it is hoped we will be able to have a wider meeting with Ngati Wairere representatives during the latter half of 2015.
**Additional Supporting Information**

The following reports are supporting information provided to expand on the previous summary 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.

**Science Report**

**Overexpression of a testes specific transgene to investigate the process of fertilization**

- Six transgenic lines were characterised for the number of transgene insertion sites. Four of the lines have a single chromosomal insertion site while the other two lines have transgene copies integrated in two sites on different chromosomes.
- Bucks from two lines were assessed for sperm production
- One buck of each of the two lines was hemicastrated
- Testes tissue samples were analysed for transgene expression and confirmed expression of the transgene for both lines
- Bucks from one line were used for natural mating with superovulated does and around 150 blastocyst and morula stage embryos were recovered on embryonic day 7 for genotypic analysis
- Female to male and transgenic to wildtype ratios in these embryos followed normal Mendelian inheritance patterns

**Overexpression of recombinant therapeutics in the milk of transgenic goats**

- Animals were farmed to maintain these transgenic lines for future development options.

**Cattle engineered for the knockdown of the milk protein beta-lactoglobulin**

- Oocytes were recovered from the tailless knockdown heifer
- Oocytes were in vitro fertilised with semen from a bull of a transgenic line expressing human lactoferrin, cultured and resulting embryos sexed, genotyped and cryopreserved
- Female embryos of the four possible genotypes were transferred to recipient for development to term and assessment of their milk characteristics

**Cattle engineered for the disruption or deletion of the gene for the milk protein beta-lactoglobulin**

- One cell embryos, produced by in vitro fertilization, were co-injected with genome editors and repair templates to introduce specific DNA sequence changes in the beta-lactoglobulin target locus
- Embryos were biopsied, characterised and cryopreserved at the blastocysts stage
- Embryos validated for the presence of the intended disruption or deletion of the beta-lactoglobulin gene were transferred to recipients for development to term

**Overexpression of bovine casein and human myelin basic protein (hMBP) in transgenic (TG) cattle**

- Animals were farmed to maintain these transgenic lines.
Overexpression of the histone demethylase KDM4B in transgenic (TG) cattle

- Fetal fibroblasts that overexpressed the histone demethylase KDM4B were used for cloning, alongside with non-induced control cells.
- Cloned blastocysts from both groups were transferred into surrogate heifers and allowed to develop until D60 before abortion.
- No difference was observed between the experimental groups, indicating that histone trimethylation is not a major stumbling block for epigenetic reprogramming in cattle.
## On Farm Management Summary for year ending 30/06/2015

### Animal Numbers 01/07/2014–30/06/2015 (Births exclude still born or animals which die soon after birth reported in Animal Ethics Reports. Aged In and Out records changes in animal age¹)

<table>
<thead>
<tr>
<th>Stock Class</th>
<th>Open (1/07/14)</th>
<th>Births</th>
<th>Transfer In</th>
<th>Transfer Out</th>
<th>Aged In</th>
<th>Aged Out</th>
<th>Killed</th>
<th>Deaths</th>
<th>Closing (30/06/15)</th>
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<tr>
<td>MA Doe</td>
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<td>27</td>
<td>13</td>
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</table>

¹ Aligns with normal livestock reconciliation aging practice.
The preceding table provides animal numbers over the reporting period in the development lines and are linked to the EPA approval. This includes transgenic and non-transgenic animals (progeny) and the conventional animals which are used to support the programmes.

For cattle; there have been two movements of conventional animals (84 in total) into the facility during the period and 2 of these moved out again as they were found to be unsuitable (temperament wise) and another 27 animals which had never held embryos.

31 cattle of varying ages have been euthanased (killed); these animals have been disposed of in offal holes on-site, having been identified as surplus or now unsuitable animals, or following veterinary advice during this period.

For goats there have been no movements of animals (apart from approved exit and returns for surgery purposes) during the period.

18 goats of varying ages have been euthanased (killed) and 5 goats died during the period; these animals have also been disposed of in offal holes on-site, as now surplus or unsuitable animals, or following veterinary advice.

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 continue to be managed in a way which is considered normal farming practice and is accepted by the majority of farmers in New Zealand.

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.

92 cattle and 56 goat recipients have been used for ET (embryo transfer) on a rotational basis during the period. Suitable goats have also been mated using non transgenic bucks to generate additional offspring, female only are normally kept, to be available as future recipients. All animals are regularly monitored for live weight and health status.

All animals have been grazed mainly on pasture, with supplementary feeding of hay or balage when required and been outside in the natural environment.

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 have access to covered shelter in inclement weather.

Surplus pasture is conserved when possible for use in periods of low growth, as balage or hay and there was only minimal purchasing of extra supplement (meal) required this season following favourable weather conditions which enabled 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 is carried out using a mineral dispensing system through the water troughs for assisting Facial Eczema control and other normal mineral deficiencies during identified periods of risk, as occurs on many farms.

Maintenance fertiliser applied this season contained nitrogen and selective additional Nitrogen (Urea) has been used on areas not used for milk/waste irrigation.
Milk Production 14/15 season

Five cows, all Casein, calved during August 2014 and 4 were milked through until mid-October 2014 when they were dried off as there was no need to keep milking and the calves were ready to be weaned off milk. One didn’t really come into milk and dried herself off within a few days of calving.

All the milk that was collected this season was fed to the calves, so none was required to be stored in 1000L IBCs until ground conditions allowed us to begin milk disposal by irrigating onto pasture within the facility, this has been based on the previous treatment method, weather and consent conditions. As identified in the table below, because of low quantities, no milk irrigation was required at all this season.

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<thead>
<tr>
<th>Cow Type/litres</th>
<th>Fate</th>
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<tbody>
<tr>
<td>Month</td>
<td>Casein</td>
</tr>
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<td>July</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>611</td>
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<tr>
<td>September</td>
<td>1144</td>
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<tr>
<td>October</td>
<td>364</td>
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<td>November</td>
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<td>December</td>
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<td>March</td>
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<td>April</td>
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<td>May/June</td>
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<tr>
<td>Season Total</td>
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</table>

The table above provides the litres of milk type collected each month on the left hand side which combines to give the figure in the ‘Total’ column. This figure will equal the combined shown in either the ‘IBC or Calves’ column. The ‘Disposal’ column equals the ‘IBC’ column and shows the amount of milk diluted and irrigated during that month.

² IBC (Industrial Bulk Container) Large Plastic container, on a pallet generally with a protective surrounding (cage) designed to hold bulk Liquids or chemicals. Various sizes up to 1000litre capacity.
Ruakura Animal Ethics Committee Reports

RAEC #13187 and 13509 - Production and characterisation of transgenic cattle, generic application

Ruakura Animal Ethics Committee Report: Third Quarter 2014

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval in relation to the condition for approval of Application 13187 ‘Production and characterisation of transgenic cattle, generic application’.

Ruakura Animal Ethics Committee Report: Third Quarter 2014

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval July to September 2014 in relation to the conditions for approval of Application 13187 (was 12618) "Production and characterisation of transgenic cattle, generic application". Approval transferred to 13187 in early April 2014.

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the founder casein animals (F0) as the oldest, being 14 years old (born 2000).

A.2 Treatments and activities during reporting interval

6008, 7017, 8023, 9005, 9015 calved and milking. 9005 difficult to milk and dried off late August. Four live calves 1431, 1432, 1433, 1434 resulted, 3 males, one female. Routine dehorning.

Routine pre calving and annual vaccinations and antiparasitic treatments. Magnesium supplementation for calvers/milkers.

A.3 Culling and losses during reporting interval

Nil. Male calf from 08023 born dead.

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

Two generations (F0 and F1) of transgenic hMBP cattle have been produced with the oldest founder hMBP animals (F0) being 13 years old (born 2002).

B.2 Treatments and activities during reporting interval

Routine annual vaccinations and antiparasitic treatments.

B.3 Culling and Losses during reporting interval

Nil
C) hLF cattle

C.1 Status of transgenic hLF cattle

No hLF cattle currently

D) Erbitux cattle

11001 3 years old and healthy. Routine annual vaccinations and antiparasite treatment.

E) Beta-lactoglobulin (BLG) knockdown (kd) cattle

11014, Daisy, one final follicular aspiration event in reporting period. Routine annual vaccinations and antiparasite treatment.

Recipients routine synchrony, mating, embryo transfer, ultrasound pregnancy testing and embryo flushing and luteolysis for various research applications. One animal treated with anti-inflammatory pain relief drugs for a hip injury (recovered).

Culling and Losses during reporting interval:
Three recipients culled due to age related lameness.

Animal Health
Routine annual vaccinations and antiparasitic treatments.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2014

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval October to December 2014 in relation to the conditions for approval of Application 13187 "Production and characterisation of transgenic cattle, generic application". Approval transferred to 13187 in early April 2014.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2014

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval October to December 2014 in relation to the conditions for approval of Application 13187 (was 12618) "Production and characterisation of transgenic cattle, generic application". Approval transferred to 13187 in early April 2014.

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the founder casein animals (F0) as the oldest, being 14 years old (born 2000).
A.2 Treatments and activities during reporting interval
6008, 7017, 8023, 9015 milkers and dried off in October (no further milk required).
1431, 1432, 1433, 1434 calves weaned, vaccinated and treated for worms.
9005, 9007, 9013, 9015, 1303 mated (AI).

A.3 Culling and losses during reporting interval
01038 euthanased for humane reasons: at 13 years she had developed arthritis.

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

Two generations (F0 and F1) of transgenic hMBP cattle have been produced with the oldest founder hMBP animals (F0) being 13 years old (born 2002).

B.2 Treatments and activities during reporting interval
1305 mated (AI).

B.3 Culling and Losses during reporting interval
Nil

C) hLF cattle

C.1 Status of transgenic hLF cattle

No hLF cattle currently

D) Erbitux cattle
11001 healthy and 3 years old.

E) Beta-lactoglobulin (BLG) knockdown (kd) cattle
11014, Daisy, no treatments.

Recipients: routine synchrony, mating, embryo transfer, ultrasound pregnancy testing and embryo flushing and luteolysis for various research applications.

Culling and Losses during reporting interval:
One recipient culled due to age related disease.

Animal Health
Routine calf vaccinations and antiparasitic treatments.

Ruakura Animal Ethics Committee Report: First Quarter 2015

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval in relation to the condition for approval of Application 13187
"Production and characterisation of transgenic cattle, generic application". Approval 13187 ended late March and project transferred to approval 13509.

Ruakura Animal Ethics Committee Report: First Quarter 2015

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval January to March 2015 in relation to the conditions for approval of Application 13187 "Production and characterisation of transgenic cattle, generic application". Approval transferred to 13509 on 19.3.15.

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the founder casein animals (F0) as the oldest, being or rising 15 years old (born 2000).

A.2 Treatments and activities during reporting interval

3061, 8025, 9007 took part in a sensor study for assessing walking (sensor placed on permanent eartag). Calves treated with poron anthelmintic.

A.3 Culling and losses during reporting interval
Nil

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

Two generations (F0 and F1) of transgenic hMBP cattle have been produced with the oldest founder hMBP animals (F0) being or rising 13 years old (born 2002).

B.2 Treatments and activities during reporting interval

2006 took part in a sensor study for assessing walking (sensor placed on permanent eartag).

B.3 Culling and Losses during reporting interval
Nil

C) hLF cattle

C.1 Status of transgenic hLF cattle

No hLF cattle currently

D) Erbitux cattle

11001 healthy and now 4 years old. Also took part in walking sensor study.

E) Beta-lactoglobulin (BLG) knockdown (kd) cattle

11014, Daisy treated with Blaze (topical deltamethrin) for fly prevention.
Recipients:
Little work involving recipients in this quarter. Synchrony of four animals with prostaglandin.

Culling and Losses during reporting interval:
Nil

Animal Health
Fly and facial eczema prevention.

Ruakura Animal Ethics Committee Report: Second Quarter 2015

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval in relation to the condition for approval of Application 13509 "Production and characterisation of transgenic cattle, generic application".

No Report submitted to RAEC at this time.

RAEC #13243 and 13585 - Development of transgenic goats in containment

AEC 13243 Interim Report – 3rd Quarter 2014

Transgenic Goats

Summarised below are activities for Application 13243 "Development of transgenic goats in Containment" for the report period.

No Report submitted to RAEC at this time.

AEC 13243 Interim Report – 4th Quarter 2014

Transgenic Goats

Summarised below are activities for Application 13243 "Development of transgenic goats in Containment" for the report period.

No Report submitted to RAEC at this time.

AEC 13243 Interim Report – 1st Quarter 2015

Transgenic Goats

Summarised below are activities for Application 13243 "Development of transgenic goats in Containment" for the report period. Approval 13243 ended late March and project transferred to approval 13585.
No Report submitted to RAEC at this time.

AEC 13585 Interim Report – 2nd Quarter 2015

Transgenic Goats
Summarised below are activities for Application 13585 "Development of transgenic goats in Containment" for the report period.

No Report submitted to RAEC at this time.

RAEC #13089 and 13433 - Development of transgenic goats showing non-Mendelian inheritance

AEC 13089 Interim Report – 3rd Quarter 2014

Transgenic Goats
Summarised below are activities for Application 13089 "Development of transgenic goats showing non-Mendelian inheritance" for the report period.

No Report submitted to RAEC at this time.

AEC 13089 Interim Report – 4th Quarter 2014

Transgenic Goats
Summarised below are activities for Application 13089 "Development of transgenic goats showing non-Mendelian inheritance" for the report period.

No Report submitted to RAEC at this time.

AEC 13089 and 13433 Interim Report – 1st Quarter 2015

Transgenic Goats
Summarised below are activities for Application 13089 "Development of transgenic goats showing non-Mendelian inheritance" for the report period. Approval 13089 ended during the period and the project transferred to approval 13433.

No Report submitted to RAEC at this time.

AEC 13089 and 13433 Interim Report – 2nd Quarter 2015

Transgenic Goats
Summarised below are activities for Application 13089 "Development of transgenic goats showing non-Mendelian inheritance" for the report period. Approval 13089 ended during the period and the project transferred to approval 13433.

No Report submitted to RAEC at this time.