

**Annual Report to
ERMA New Zealand
for
Activities under ERMA 200223
AgResearch Ltd**

30th June 2011

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Contents

Summary of Activities	Pg3
Outdoor Development Activities	Pg 3
Unforeseen adverse effects	Pg 3
ERMA200223 Liaison Group	Pg 3
Science Report	Pg 4
On Farm Management Summary	Pg 6
Ruakura Animal Ethics Committee Reports	Pg 9
MAF Biosecurity New Zealand Audit reports	Pg 18

Summary of Activities for the period 1st July 2010 to 30th June 2011

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.

Goats developed under the GMD09016 approval are being maintained under the ERMA200223 approval and all further/ ongoing goat development activities are now being undertaken under this approval.

Cattle and Goat activities, other than the maintenance or growing of animals, have been the calving and milking of cows, kidding of recipient goats, 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.

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 met twice.

An initial establishment meeting was held in November 2010 which included wider Ngati Wairere and Tainui representation along with Kaupapa Kura Taiao representatives; following an update on the science and some background information, we worked through an initial terms of reference outline and representation requirements.

The draft terms of reference were circulated prior to the second meeting on the 1st of April 2011 at which they were further discussed and refined.

While not fully confirmed at this stage the terms of reference outline similar principles which the previous monitoring group (GMD02028/GMF98009) had endorsed.

A joint information and facility visit meeting with Ngā Kaihautū Tikanga Taiao was held on the 1st of June 2011 which was beneficial to all attending.

A further meeting of the liaison group has been scheduled for September 2011 following scheduling difficulties and key attendees availability.

Science Report

Overexpression of a monoclonal antibody in the milk of transgenic goats

- Transgenic cells containing gene constructs for the expression of the immunoglobulin (IgG) light- and heavy-chains were used to reconstruct goat embryos using nuclear transfer.
- The transgenic embryos were transferred into recipient goats for development to term.
- Kids born from the first two transgenic lines were genotypically female but phenotypically male.
- The cause for this sex reversal was identified to be the naturally occurring mutation for the polled intersex syndrome (PIS) which was carried by the parental cell line used to generate the two clonal transgenic lines and is not caused by the genetic modification.
- Histological analysis of the testis from these males showed no signs of sperm production indicating that these animals were sterile.
- Kids born from the third transgenic line, which did not carry the PIS mutation, were normal females.
- Detection of transgene specific DNA sequences within the genome by Southern analysis indicated that both transgenes were truncated (shortened) in this third transgenic line.
- Kids of lines 1 and 3 were successfully induced into lactation, while we were unable to produce milk from line 2 kids.
- Immuno-detection of human IgG in goat milk by Western analysis revealed the production of transgene encoded human IgG in the milk produced by kids from line 1.
- Line 3 kids did not appear to produce any human IgG in milk which is consistent with the detection of truncated (shortened) transgenes in this line.
- Reconstructed embryos from six further clonal lines (lines 4-9), each verified for the presence of intact transgenes, were transferred into recipients for development to term.
- Kids from transgenic line 4 have been born and can soon be tested for human IgG production.
- Pregnancies for transgenic lines 5-9 are at different gestational stages with the next kids due to be born in August 2011.

Overexpression of a monoclonal antibody in the milk of transgenic cattle

- Following transfer of transgenic embryos, reconstructed with a transgenic bovine cell line for production of a monoclonal antibody in milk, one transgenic calf was produced.
- The molecular characterisation revealed the presence of the transgene encoding the light chain, while the heavy chain construct could not be detected.

Overexpression of human follicle stimulating hormone (hFSH) in transgenic hFSH cattle

- After Veterinary advice, the hFSH cow was euthanised at the completion of the project after cells were isolated from an ear biopsy of the hFSH cow, frozen and stored for potential future use of this transgenic line.

Overexpression of bovine casein in transgenic (TG) Caseinplus cattle

- No development activities

Overexpression of human myelin basic protein (hMBP) in transgenic hMBP cattle

- Milk was sampled, tested for hMBP expression and freeze dried. Freeze dried milk powder was sent to our collaborators for purification and functional testing of hMBP.

Overexpression of human lactoferrin (hLF) in transgenic hLF cattle

- Milk yields of the hLF heifers are as expected and consistent with previous results.

Rejuvenation of transgenic cell lines by re-deriving cell lines from nuclear transfer foetuses

- Cell lines engineered with various different constructs, including constructs for the expression of a monoclonal antibody, a micro RNA and the fluorescent protein EGFP, were used to produce early stage foetuses for recovery and re-derivation of cells with renewed growth potential.

On Farm Management Summary for year ending 30/06/2011

Animal Numbers 01/07/2010– 30/06/2011 (Births exclude still born or animals which die soon after birth reported in Animal Ethics Reports)

Stock Class	Open (1/07/10)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/11)
Casein (ERMA200223)									
MA Cows	38				11		3		46
R2yr Heifers	11				6	11	1		5
Spring R1yr Heifers	6				0	6			0
Spring Heifer Calves	0								0
R2yr (+) Bulls	11				3		1		13
R1yr Bulls	3					3			0
Spring Bull Calves	0								0
Total Casein	69	0	0	0	20	20	5	0	64
MBP (ERMA200223)									
MA Cows	6						1		5
R2yr Heifers	0								0
R1yr Heifers	0				1				1
Heifer Calves	0	1				1			0
2yr+ Bulls	1								1
R1yr Bulls	0								0
Total MPB	7	1	0	0	1	1	1	0	7
rhLF (ERMA200223)									
MA Cows	8				6				14
R2yr Heifers	6				0	6			0
R1yr Heifers	0				4				4
Heifer Calves	0	4				4			0
2yr (+) Bulls	4						4		0
R1yr Bulls	1				6				7
Bull Calves	0	6				6			0
Total rhLF	19	10	0	0	16	16	4	0	25
FSH (ERMA200223)									
R2yr Heifers	2						2		0
R1yr Heifers	0								0
Total FSH	2	0	0	0	0	0	2	0	0
Erbitux (ERMA200223)									
Aut Hfr Calves		1							1
Total Erbitux	0	1	0	0	0	0	0	0	1
Conventional Cattle									
MA Cows	71		7	2			11		65
Aut Hfr Calves			1						1
Total Conventional	71	0	8	2	0	0	11	0	66
Cattle Total	168	12	8	2	37	37	23	0	163
Cattle developed under ERMA approvals (Tg and non Tg progeny)									97
Goats									
Erbitux (ERMA200223)									
R1yr Doe					7				7
Doe Kid		4	4			7		1	0
R1yr Buck					16		16		0
Buck Kid			17			16		1	0
Total Erbitux	0	4	21	0	23	23	16	2	7
Conventional Goats									
MA Doe			36				11	1	24
Two tooth Doe					26				26
R1yr Doe			26		14	26	1		13
Doe Kid			14			14			0
Total Conventional	0	0	76	0	40	40	12	1	63
Goat Total	0	4	97	0	63	63	28	3	70
Goats developed under ERMA approvals (Tg and non Tg progeny)									7

The preceding table shows Animal numbers over the period linked directly to ERMA approvals; (includes transgenic and non transgenic animals) and the conventional animals which are used to support the programmes.

For Cattle; there have been two movements of conventional animals (8 in total) into the Facility and one (2 animals) out during the period.

23 cattle of varying ages have been euthanased (killed); these animals have been disposed of in offal holes on-site, following ethics approval to dispose of surplus or now unsuitable animals, or following veterinary advice during this period.

For Goats there have been seven movements of animals (21 Tg goats from indoor facilities and 76 conventional goats, also from indoor facilities) during the period.

28 goats of varying ages have been euthanased (killed) and 3 goats died naturally during this period; these animals have been disposed of in offal holes on-site, as now surplus or unsuitable animals, or following veterinary advice.

For management purposes 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.

Over the period all animals on the Facility have continued 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.

69 conventional Recipients have been used for ET (embryo transfer) on a rotational basis during the period. 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 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 the purchasing of extra supplement was again required during the season following challenging weather conditions to maintain an adequate annual feed supply.

The majority of the pastures now available on the Facility are not Ryegrass based or are endophyte free Ryegrass and regular pasture renewal is standard practice, with nearly all paddocks being in new grass at least once since the facility was established.

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.

Nitrogen, as Urea, was applied to the whole (45ha) facility at 20kgN/ha during August 2010, as part of a spring fertiliser dressing to increase available pasture levels.

Milk Production 10/11 season

Twelve cows, Ten rhLF and two MBP, have calved from late July through until October 2010 and were then milked through until mid February when they were dried off.

All the milk that was not being fed to calves was 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.

Cow Type/litres				Fate			
Month	Casein	rhLF	MBP	Total	IBC ¹	Calves	Disposal
July		61		61	49	12	
August		3281	1040	4321	2980	1341	
September		4645	1209	5854	2774	3080	1250
October		5263	1174	6437	2717	3720	3000
November		7682	989	8671	5451	3220	4750
December		5153	696	5849	3749	2100	3000
January		4788	669	5457	5457		5000
February		2809	402	3211	3211		3500
March							4000
April							1888
May/June							
Season Total		33682	6179	39861	26388	13473	26388

As in previous seasons treatment of the milk prior to irrigating was by natural fermentation which had dropped the pH of the stored milk to a level of 4 or under (acidic).

All of the balance of stored and treated milk was able under the resource consent conditions to be irrigated during March and April completing milk disposal for the season.

¹ 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 #11687 Production and characterisation of transgenic cattle, generic application

Ruakura Animal Ethics Committee Report: Third Quarter 2010

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 RAEC#11687 Production and characterisation of transgenic cattle, generic application.

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 10 years old.

A.2 Treatments during reporting interval

03061 remained grazing with heifers, and healthy with this peer group.
09011, casein homozygous bull, remains smaller than peers, but is apparently healthy.
06003 treated for lameness (successful)

A.3 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 8 years old.
Two cows calved during the reporting interval and are both being milked. One of the calves was born dead; the other one is alive and healthy.

B.2 Treatments during reporting interval

07008 treated for mastitis post calving (successful).

B.3 Losses during reporting interval:

One dead calf at birth, see above

C) hLF cattle

C.1 Status of transgenic hLF cattle

Founder animals (F0) of transgenic hLF cattle have been produced with the oldest founder hLF animals being rising four years old. 6 animals calved and are milking. All progeny are well.

C.2 Treatments during reporting interval

06041 and 07001 treated for mastitis (successful).

C.3 Losses during reporting interval: Nil

D) Erbitux cattle

Two nuclear transfer (NT) runs were undertaken end of August with a cell line rederived from a transgenic foetus. The embryo transfers failed to result in pregnancies past d42. Transfer of vitrified NT embryos resulted in two good pregnancies, currently at d132. The recipient from an earlier embryo transfer with the most advanced pregnancy (d236) showed signs of accumulating fluid and is now closely monitored.

Health activities involving transgenic or non transgenic cattle

All adult animals had their annual vaccination for leptospirosis and clostridial disease.

The recipients had an annual vaccination for neospora.

Calvers were vaccinated for rotavirus and E.coli.

All animals were routinely treated with vitamin B12 and selenium.

All calves (7) were dehorned and given their initial clostridial vaccination.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2010

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 RAEC#11687 Production and characterisation of transgenic cattle, generic application.

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 10 years old.

A.2 Treatments during reporting interval

05030, 07007, 07010 all treated for dermatophilus on legs

06003 sick, unilateral facial paralysis, thoracic pain, lack of competition with peers

A.3 Losses during reporting interval:

06003 euthanased 29.10.10, due to lack of response to treatment and ongoing facial paralysis and ill thrift.

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 8 years old.

B.2 Treatments during reporting interval

02054 treated for lameness in front leg

B.3 Losses during reporting interval: Nil

C) hLF cattle

C.1 Status of transgenic hLF cattle

Founder animals (F0) of transgenic hLF cattle have been produced with the oldest founder hLF animals being rising four years old.

C.2 Treatments during reporting interval

06041 treated for grass staggers (hypomagnesaemia)
06023, 06038, 06045 & 08002 calved, all calves healthy and cows milking

C.3 Losses during reporting interval:

06034, 06037, 06042 and 06046 euthanased 16.11.10 (males, surplus to requirements).

D) hFSH cattle

D.1 Status of transgenic hFSH cattle

08034 - after regular scanning of her ovaries it was decided that the persistent enlarged cystic bodies on the ovaries prevented safe aspiration of follicles, and that large size and heavy weight of the ovaries meant there was a continuing risk of rupturing vascular structures by manipulating the ovaries. It was regrettably decided that 08034 should be euthanased on welfare grounds. Euthanasia on 14.10.10 was carried out using an overdose of sodium pentobarbitone. Alan Julian (Gribbles Veterinary Pathology) carried out a post mortem examination, the report is attached to AE Application 12074 as pdf 2920. 08031 was euthanased for science (paired with 08034 from birth) on 27.10.10.

D.2 Treatments during reporting interval

Scanning followed by euthanasia as above.

D.3 Losses during reporting interval: Planned euthanasia, both animals.

E) Erbitux cattle

Transfer of vitrified NT embryos resulted in two good pregnancies (Erbitux group 50), at d 221 on 28.12.10. Recipient cows scanned regularly and monitored carefully for excess abdominal fluid (hydrops pregnancy).

The recipient from an earlier embryo transfer (Erbitux Group 46) started to show clinical hydrops on 5.10.10 (day 241 of pregnancy), she was induced and the calf delivered by caesarean on 17.10.10 (day 252 of pregnancy). The calf was premature (30 days) and only survived for 2 hours. It had a swollen abdomen typical of hydrops calves, and respiratory

difficulty. The dam was euthanased, and the calf post-mortemed.

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

Two embryo transfer (ET) runs were undertaken 14th and 16th of December (BLG kd Group 53 and 54) with NT-embryos produced with a cell line containing an miRNA expression construct targeting BLG expression. The first ultrasound scans are scheduled for January 2011.

Activities involving transgenic cattle

Mating, natural and AB, of selected cows. Routine treatment for anoestrus cows required for mating.

Pharming calves dehorned and vaccinated for clostridial disease.

Ruakura Animal Ethics Committee Report: First Quarter 2011

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 RAEC#11687 Production and characterisation of transgenic cattle, generic application.

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 11 years old.

A.2 Treatments during reporting interval

06012 treated for lump on side of face, 07010, 09011 treated for dermatophilus, 08029 treated for woody tongue, 09013 treated for lump on face. 01038, 01012, routine ear biopsies taken.

A.3 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 9 years old.

B.2 Treatments during reporting interval

02054 failed to recover from lameness in front leg. Euthanased on welfare grounds, post mortem revealed degenerative arthritis in shoulder joints (reason for lameness). This was probably a normal consequence of ageing.

B.3 Losses during reporting interval

02054 as detailed in B.2

C) hLF cattle

C.1 Status of transgenic hLF cattle

Founder animals (F0) of transgenic hLF cattle have been produced with the oldest founder hLF animals being rising 5 years old.

C.2 Treatments during reporting interval: Nil

C.3 Losses during reporting interval: Nil

D) Erbitux cattle

Two cows pregnant for group 50 reached last month of pregnancy. One suffered hydrops and delivered a dead calf pre term, the second delivered a live heifer calf, 01011, aka Erbie on 18.2.11.

This calf is now 6 weeks old and thriving.

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

Two embryo transfer (ET) runs were undertaken 14th and 16th of December (BLG kd Group 53 and 54) with NT-embryos produced with a cell line containing an miRNA expression construct targeting BLG expression. The first ultrasound scans in January showed some pregnancies, but these all failed by day 52.

A further two ET runs were carried out on 25.2.11 and 1.3.11. Preliminary scans on 29.3.11 showed 5 pregnancies.

Activities involving transgenic cattle

Milking cows dried off mid February, routine worming of calves.

Ruakura Animal Ethics Committee Report: Second Quarter 2011

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 RAEC#11687 'Production and characterisation of transgenic cattle, generic application'.

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 11 years old.

A.2 Treatments during reporting interval

09011 (homozygous casein bull) treated again for dermatophilus, and ability to produce semen assessed.

07004 also treated again for dermatophilus.

05033 routine ear biopsy taken, euthanased due to chronic arthritis.

06031 squamous cell carcinoma right third eyelid ? removed.

08026 abscess lower right jaw.

09013 lump lower right jaw. Treated with antibiotics.

09006, 09011 euthanased, chronic small size and illthrift compared with peers under same husbandry regime. No abnormalities found on post mortem. Histological examination of 09011's testicles confirmed absence of sperm production.

A.3 Losses during reporting interval:

05033, 09006, 09011 as detailed in A2

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 9 years old.

B.2 Treatments during reporting interval: Nil

B.3 Losses during reporting interval: Nil

C) hLF cattle

C.1 Status of transgenic hLF cattle

Founder animals (F0) of transgenic hLF cattle have been produced with the oldest founder hLF animals being rising 5 years old.

C.2 Treatments during reporting interval

06047, aborted ~ 4 month pregnancy, no infectious causes detected on pathological examination.

C.3 Losses during reporting interval: Nil

D) Erbitux cattle

11001 has been dehorned and had routine calfhood vaccinations for leptospirosis and clostridial disease.

She is growing well, and had one incidence of rumen dysfunction associated with a chronic bacterial infection in late June. This responded to antibiotic treatment.

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

Group 55/56 transfers in February resulted initially in one (55) and four (56) pregnancies.

At the end of June, the one Group 55 and one of 56 pregnancies remain, now at day 120 of pregnancy. Permission has been granted from the Ethics Committee to carry these pregnancies to term with weekly monitoring for hydrops/other problems affecting maternal health.

Activities involving transgenic cattle

All animals received annual leptospirosis vaccination and routine worming/delousing prior to winter.

RAEC #11690 Development of transgenic goats in containment

Ruakura Animal Ethics Committee Report: Third Quarter 2010

Please see the attached pdf that provides a summary of pregnancy and embryo survival data in this trial to date, following the transfer of 1- to 2-cell stage cloned-transgenic embryos to the oviducts of recipient does. (pdf withheld as it contains confidential indoor development related information).

We have had our first "batch" of cloned-transgenic kids born. A total of 12 kids were delivered at term, with 10 surviving (2 died at birth). (Please refer to the Veterinarian Note dated 10.09.10). Unfortunately, although these kids are genetically female (as intended), they are in fact phenotypically male. This intersex phenotype arose from the day 38 fetus, used to derive the cell lines for this first batch of embryo transfers, being homozygous for the naturally occurring deletion in the PIS (polled intersex) locus. We now appreciate the prevalence of this deletion in the former GTC Whatawhata goat herd.

The same fetal cell line was used for the second "batch" of embryo transfers; these kids are due in October. Therefore, they too will display the intersex phenotype. We have just determined by PCR that the third "batch" of kids, due in November and derived from a different female fetal cell line, possess a wild type copy of the PIS allele. Therefore, these kids should indeed be phenotypic females. We are presently screening other cell lines we have in storage for the PIS deletion. In future, we will only derive cell lines from horned goats to avoid this problem.

Veterinarian Note: (10/09/2010) referred to in above report.

An update on our goat kidding which started this week.

We now have: 91 - twins born 7.9.10 (day 146)

10 - single born 7.9.10 (day 146)

35 - triplets (1 dead at birth) born 10.9.10 (day 145)

111 - QUADS!! (first breach, eyes dilated at birth and died shortly after) born 10.9.10 (day 145)

There are two animals (51 and 84) left to kid on Tuesday 14th September.

So, we currently have 8 alive Toggenberg intersex clones. All are outwardly anatomically normal males apart from one small twin from 91 which has a caudally placed preputial structure (just in front of testicles and odd shape).

Unfortunately the intersex was not part of the plan. These animals are genetically female (have 60XX chromosomes). However they are (quite by chance) derived from a homozygous polled foetal cell line, this means that they then express a recessive gene for intersex.

The first two kids born have been confirmed to be genetically female and also to carry both transgenes which were inserted.

We will post mortem the two dead kids to see what internal gonadal structures they have. The kids due later in the programme are from a different foetal cell line, so may be normal females.

The live kids are otherwise normal in appearance, anatomy and behaviour. They are vigorous and feeding well.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2010

Please see the attached pdf that provides a summary of pregnancy and embryo survival data in this trial up to 31st December 2010, following the transfer of 1- to 2-cell stage cloned-transgenic embryos to the oviducts of recipient does. (pdf withheld as it contains confidential indoor development related information).

Ruakura Animal Ethics Committee Report: First Quarter 2011

ETs were performed in Groups 8 & 9 in late November / early December 2010, using a cell line designed GL6.7. Seven of the 18 recipients were initially diagnosed pregnant at Day 30 of gestation. Five recipients remain pregnant and are currently between Days 111-114 of gestation. Kidding is scheduled the week after Easter. One of these does is expected with triplets, with the other four carrying singletons. The overall embryo survival rate for these two groups is 6.2% (seven fetuses from 113 one- and two-cell embryos that were transferred).

Recently, we performed additional transfers of NT embryos (Groups 10 and 11) reconstructed with two new cell lines (GN118 and GN100, respectively). In Group 10, a total of 100 one- and two-cell embryos were transferred into 12 recipients. In Group 11, a total of 77 one- and two-cell embryos were transferred into 10 recipients. The first scan results will be available at around Day 30.

Ruakura Animal Ethics Committee Report: Second Quarter 2011

In groups 8 and 9 (cell line GL6.7), five recipients were pregnant in the third trimester. One of these (doe #29) was pregnant with triplets. Unfortunately she was euthanased on 3.04.11, at 122 days of gestation. She was an older doe (84kg liveweight) with bilateral arthritis in the stifle (and hip) joints (confirmed by post-mortem) accounting for her discomfort when standing. Combined with the weight of her uterus in late gestation (~30kg) and udder (11kg), she was increasingly unable to get up and walk comfortably. Despite extra nursing and supplemental feed, her condition declined over the preceding weeks and a decision was made by Ali Cullum to euthanase her. Three kids were examined upon post-mortem – all in good condition and anatomically normal for the stage of gestation.

The four remaining does in groups 8 and 9 were kidded in the week after Easter. All delivered single, female kids (two by Caesarean section). However, one kid (#1104) died after one day, on 29.04.11. It was observed that the kid's abdomen swelled up and she became increasingly distressed after suckling, with no evidence of passage of faeces after the first 24 hours. The kid underwent an abdominal x-ray and ultrasound examination. This revealed an enlarged abomasum and some faecal matter in the cranial intestine. No faecal matter in the caudal intestine was visible.

It was then decided to perform an exploratory laparotomy. No gross abnormality of the abomasal outlet (pylorus), or of the caudal gastro-intestinal tract was evident. The laparotomy wound was closed and treatment for pain relief and medical management of the condition given. However, the kid failed to wake up from anaesthetic. Breathing on oxygen

was maintained for two hours after the operation finished. But when the kid was finally removed from the anaesthetic machine it did not resume voluntary respiration and died shortly afterwards.

Post-mortem examination revealed a lot of partially clotted and digested milk in the rumen, reticulum and omasum (forestomachs) in addition to the abomasum. The oesophageal groove was present, but shallow in structure. In conclusion, it was considered that the acute abdominal pain and distension was due to a failure of oesophageal groove closure and / or failure of abomasal emptying.

The overall embryo survival rate for groups 8 and 9 reduced from 6.2% at the beginning of the third trimester (7 fetuses / 113 one- and two-cell embryos that were transferred), down to 2.7% (3/113) in terms of viable kids at term (with the loss of doe #29 and kid #1104). The three remaining kids are healthy at 2 months of age and will be induced into lactation in another couple of months.

In group 10 (cell line GN118), only one doe from 12 recipients (receiving a total of 100 1- and 2-cell embryos) initiated a pregnancy at Day 30; and she remains pregnant with a singleton, currently at 114 days of gestation. Overall, this represents 1% embryo survival (1/100) with this particular cell line.

In group 11 (cell line GN100), four does from 10 recipients (receiving a total of 70 1- and 2-cell embryos) initiated pregnancies at Day 30 and all four remain pregnant, currently at 111 days of gestation. Three does are carrying singletons and one has twins. Overall, this represents 6.5% embryo survival (5/77) at this stage of development.

In group 12 (cell line GL8.3), we transferred of 54 NT embryos into 10 recipients. Three does are currently pregnant with a total of 4 fetuses, currently at Day 58 of gestation. This represents an embryo survival of 7.4% (4/54) at the end of the first trimester.

Recently, in groups 13 and 14, we performed additional transfers of NT embryos reconstructed with two new cell lines (GN97 and GN99, respectively). In Group 13, a total of 25 one- and two-cell embryos were transferred into 5 recipients. In Group 14, a total of 26 one- and two-cell embryos were transferred into 6 recipients. The first scan results will be available at around Day 24.

In addition, as we had surplus recipients that had been synchronised from runs 13 and 14, we decided to transfer some frozen / thawed conventional Saanen x Nubian goat embryos in an opportunity to breed some more replacement females for our CAE-free herd on the Animal Containment Unit. A total of 35 morula and blastocyst-stage embryos were transferred into 12 recipients. We await scanning results in another fortnight.



Animal Containment Farm AgResearch

Facility Code: 2893

Date of Inspection: September 10 2010

Biosecurity Inspector: Crystal Lange Accompanied by Susan Thomson (ERMA)
Location: ACF, AgResearch, Ruakura Research Centre, Hamilton

Scope

To audit compliance with the MAF Biosecurity New Zealand Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals.

References

MAF BNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals

Facility Containment manual v 7.1.1 draft (Approved 10/09/10)

ERMA Approval ERMA200223 (Current)
GMF98009 and GMD02028 (historic)

Background

The MAFBNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals specifies the structural and operating requirements for field testing of farm animals in containment. This standard applies to farm animals that are classed as new organisms.

It specifies how the Director General may approve these facilities and their operators. This standard is approved by the Environmental Risk Management Authority (ERMA) in accordance with the Hazardous Substances and New Organisms Act 1996 (HSNO). ERMA will assess individual projects for approval within these containment facilities, and then may issue further controls additional to this standard.

The facility at AgResearch Ruakura is approved, under section 39 of the Biosecurity Act 1993, for the holding of farm animals that are new organisms. As part of the approval process, the facility has nominated a person responsible for the facility who has been approved as the operator pursuant to section 40 of the Biosecurity Act 1993.

The audit is to determine whether the structural and operational requirements of MAFBNZ Standard 154.03.03 are being fulfilled. This report outlines the actions (if any) AgResearch Ruakura need to take to comply with the above Standard.

Audit comments

The Facility animal register was reviewed, a physical stock count undertaken and containment measures assessed. Internal audit and transfer records were sighted.

Key findings

The following quotes in italics are taken from the MAF Biosecurity New Zealand Standard: 154.03.06 Containment Standard for the Field Testing of Farm Animals and is a selection of the areas assessed at the audit.

3.4 Training (excerpt)

Training must be available to all staff and service people. Document training records for all staff.

Facility records for staff training were not assessed as a delay had been approved by the previous Supervisor to allow for the facility manual to be revised following transition between ERMA Approvals. Training will be scheduled following approval of the manual.

3.5 Internal Controls

All audit and review findings and any corrective actions that arise from them shall be documented

The July internal audit was supplied for review shortly after it was conducted. No issues were raised.

3.6 Version

Record the version number and issue date of the containment manual on each page. Updates are to be approved by the supervisor who shall also hold an up to date copy of the manual.

The Manual was approved the morning of the audit and formally notified to the facility on September 14 2010.

4.2 Site And Fencing Requirements

The facility shall be constructed and operated in a manner to ensure that the farm animals are securely contained within the facility and isolated from other non-containment farm animals

During a tour of the site, perimeter fencing was inspected in three locations, and other sections viewed for a distance. The facility had not noted any issues with the fencing or general security of the facility.

The perimeter fence is usually checked weekly as part of routine staff activities. The day book notes some of these checks. The frequency of perimeter checks and recording of the check is not noted in the manual. As such it appears the check is only recorded if a staff member comes in specifically to do this.

4.5.1 Register

A register of all farm animals shall be maintained. The following records shall be kept for each farm animal:

- *a unique identification number,*
- *the breed, the identity of the dam and sire, sex and date of birth,*
- *details of genetic modification, if any,*
- *date of death and cause (where known).*

'Animal Tools' was accessed, mob records were reviewed. And a selection of calves traced back to the original surrogate or true ancestor.

4.5.2 Identification

All farm animals shall be permanently and individually identified by at least two approved methods. These include a coloured plastic tag, a metal tag, a tattoo, and a microchip.

All sighted animals were appropriately labeled, identification methods are recorded in the register.

4.5.3 Farm animal count (abridged)

The operator shall, during the normal day to day farm operations, actively count and record the number of animals in a mob ... This regular check shall be used to provide evidence to the supervisor that the farm animal numbers are being monitored.

A stock count was undertaken during the audit as required in the facility manual. A discrepancy between the actual and hard copy printout proved to be a technical error where the transfer of non GM cattle off site had not been saved. Several staff were aware of the transaction and an updated stock record was provided following the audit.

4.9 Vermin Control

The containment manual or quality system shall describe how vermin shall be kept to a minimum level, how surveillance for their presence is maintained and what control activities will be undertaken if they are detected.

Basic pest control is supplied by Ecolab through the Site Management, the facility Operator is advised of visits/findings. Larger vermin are controlled by facility staff as required.

Summary of Corrective Actions / Recommendations

Corrective actions (a corrective action request for a non-compliance that is not a serious risk to biosecurity. Work will be permitted to continue but the facility will be given a specified period of time to rectify the non-conformity, e.g. procedures for access are not available at the entrance.)

There were no corrective actions issued as a result of this visit

Recommendations (The following comments are recommendations only. They are non binding and are noted to assist you to enhance your systems to maintain compliance with the requirements of the standard. They are based on observations made by the auditor during the course of the audit.)

All perimeter checks be recorded to maintain consistent records. The record would verify the check is undertaken and prevent a check being missed if staff members are away or daily jobs are reprioritised.

Thank you for your time during my review of your facility. On the basis of my audit of your containment facility on the 10th September 2010, I am satisfied that you are currently operating in compliance with the MAFBNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals. Therefore I am recommending to MAF Biosecurity New Zealand that they continue your approval to this Standard.

Yours sincerely,



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Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

MAF BIOSECURITY NEW ZEALAND Border Standards

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Animal Containment Farm

AgResearch

Facility Code: 2893

Date of Inspection: April 27 2011

Biosecurity Inspector: Crystal Lange

Location: Ruakura Farm, AgResearch, Ruakura Research Centre, East St, Hamilton

Scope

To audit compliance with the MAF Biosecurity New Zealand Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals.

References

MAF BNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals

Facility Containment manual v 7.1.1

ERMA Approval ERMA200223

Background

The MAFBNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals specifies the structural and operating requirements for field testing of farm animals in containment. This standard applies to farm animals that are classed as new organisms.

It specifies how the Director General may approve these facilities and their operators. This standard is approved by the Environmental Risk Management Authority (ERMA) in accordance with the Hazardous Substances and New Organisms Act 1996 (HSNO). ERMA will assess individual projects for approval within these containment facilities, and then may issue further controls additional to this standard.

The facility at AgResearch Ruakura is approved, under section 39 of the Biosecurity Act 1993, for the holding of farm animals that are new organisms. As part of the approval process, the facility has nominated a person responsible for the facility who has been approved as the operator pursuant to section 40 of the Biosecurity Act 1993.

The audit is to determine whether the structural and operational requirements of MAFBNZ Standard 154.03.03 are being fulfilled. This report outlines the actions (if any) AgResearch Ruakura need to take to comply with the above Standard.

Audit comments

The Facility animal register was reviewed, a sampled physical stock count undertaken and containment measures assessed. Internal audit, training and transfer records were sighted.

Key findings

The following quotes in italics are taken from the MAF Biosecurity New Zealand Standard: 154.03.06 Containment Standard for the Field Testing of Farm Animals and is a selection of the areas assessed at the audit.

3.4 Training *(excerpt)*

Training must be available to all staff and service people. Document training records for all staff.

Training had been conducted following approval of the 2010 version of the manual.

3.5 Internal Controls

All audit and review findings and any corrective actions that arise from them shall be documented

No were issues noted at the December internal audit. The electronic copy was sighted.

4.2 Site And Fencing Requirements

The facility shall be constructed and operated in a manner to ensure that the farm animals are securely contained within the facility and isolated from other non-containment farm animals

The perimeter fence is checked as part of routine staff activities, this check is noted in the day book. There have been no issues with containment for either the cattle or goats held on site.

The goats are being suitably contained within the cattle barrier (Appendix 2 of the Standard) as such modification of the existing fence is not required.

4.5.1 Register

A register of all farm animals shall be maintained. The following records shall be kept for each farm animal:

- *a unique identification number,*
- *the breed, the identity of the dam and sire, sex and date of birth,*
- *details of genetic modification, if any,*
- *date of death and cause (where known).*

‘Animal Tools’ was accessed, mob records were reviewed. Selections of mobs counted during the farm tour were verified electronically and by site staff.

4.5.4 Genetic Material

A register of stored genetic material shall be maintained by the operator. Records shall include:

- *the identity of donors and recipients,*
- *number of embryos and straws of semen,*
- *dates of collection and implantation and insemination*

Goat records were assessed at this visit. The health register records all veterinary treatments. Activities in relation to animal cycling and pregnancy scans were noted, record embryo transfer was not. Information on the dates of ET and contributing cell lines and modifications for kids born the day of the audit were saved in an electronic file that was difficult to locate.

4.9 Vermin Control

The containment manual or quality system shall describe how vermin shall be kept to a minimum level, how surveillance for their presence is maintained and what control activities will be undertaken if they are detected.

Basic pest control is supplied by Ecolab through the Site Management; a recent control shoot for rabbits was noted in the day book.

Summary of Corrective Actions / Recommendations

Corrective actions *(a corrective action request for a non-compliance that is not a serious risk to biosecurity. Work will be permitted to continue but the facility will be given a specified period of time to rectify the non-conformity, e.g. procedures for access are not available at the entrance.)*

There were no corrective actions issued as a result of this visit

Recommendations *(The following comments are recommendations only. They are non binding and are noted to assist you to enhance your systems to maintain compliance with the requirements of the standard. They are based on observations made by the auditor during the course of the audit.)*

Enter all relevant date into the health record or clearly link the entry to files containing the relevant information.

Thank you for your time during my review of your facility. On the basis of my audit of your containment facility on the 27th April 2011, I am satisfied that you are currently operating in compliance with the MAFBNZ Standard 154.03.06 Containment Standard for the Field Testing of Farm Animals. Therefore I am recommending to MAF Biosecurity New Zealand that they continue your approval to this Standard.

Yours sincerely,



Crystal Lange
Biosecurity Inspector
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