

THE WINSTON CHURCHILL MEMORIAL TRUST

CHURCHILL FELLOWSHIP 2007

BRAD PULLEN



The Samuel and Eileen Gluyas Churchill Fellowship to study commercial IVF techniques with a view to integrating these techniques into the Australian cattle industry.

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Signed: Brad Pullen

Dated 15/03/2009

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Introduction

The Australian beef industry is a significant one for rural and regional Australia. Furthermore it is an export orientated industry with approximately 70% of total product currently exported. Until recently Australia was the world's largest exporter of beef having just been overtaken by Brazil. Part of Brazil's success in the world beef market has been Brazil's rapid research and adoption of new technologies. In particular, new technologies associated with reproduction in cattle have received prominence. The main Advanced Assisted Reproductive Technology (AART) that Brazil is adopting and pioneering is that of, In-Vitro Production (IVP) of embryos.

The Churchill Fellowship allowed me to study Advanced Assisted Reproductive Technologies (AART), particularly In-Vitro Production of cattle embryos, in Brazil and The USA and further investigate its application to the beef industry.

Acknowledgements

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Further-more I cannot thank enough those persons integral to the facilitation of The Samuel and Eileen Gluyas Fellowship, for their self-less donation.

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Andreas Sobalvarro Ruata	Cabanha Libre, Brazil
Marianna Sobalvarro Ruata	Cabanha Libre, Brazil
Dr Carlos Zenenga	Embriza, Brazil
Dr Pietro Baruselli	University of Sao Paulo, Brazil.
Tony Brown	Pioneer Park, Australia.
David Deguara	Simla, Australia.
Dr Wayne Hall	MLA, Brisbane
Dr Scott Norman	Charles Sturt University
Dr Guilherme Lemos	Embriza, Brazil.

Executive Summary

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Fellowship Project

This project involved the evaluation of Advanced Assisted Reproductive Techniques (AART) as they apply to the cow and their possible incorporation into the Australian cattle industry with particular emphasis on the northern or tropical beef cattle industry. Travel was undertaken from the 5th March to the 5th April 2008. I specifically visited the laboratory of Dr Peter Hansen to learn the techniques of In Vitro Production of embryos. During this time I was able to practice these techniques and pick-up valuable hands on experience. The Brazilian leg of the fellowship allowed me to see multiple functioning commercial IVF laboratories providing much wanted IVF services to the Brazilian cattle industry and again obtain hands on experience.

Fellowship Highlights

- Dairy Research Unit University of Florida. Cutting edge research program into IVP of embryos.
- Synergy IVF Lab Texas. Commercial IVP Laboratory
- Houston Livestock Show and Sale.
- Cabanha Libre, Campo Grande, Brazil. Brazilian “ranch” using the IVF technology.
- Embriza, Brazil. Commercial Brazilian cattle IVF facility.

Major Findings, Dissemination and Implementation

This study tour clearly revealed that IVP is an established practical reproductive technology being utilized in the Brazilian and U.S. cattle industries. I believe this particular technology will play an important role in the Australian cattle breeding sector due to close industry similarities between Brazil, the U.S. and Australia.

The dissemination of the information collected will be by interviews on ABC Radio, contacts already in the cattle breeding business, conferences with the Australian Reproduction Veterinarians, local newspaper articles on the Churchill Fellowship and involvement in various cattle breed associations. Implementation of these techniques could be mediated through universities especially James Cook in north Queensland. The major method of dissemination will be via practically implementing these techniques myself in Australia.

Fellowship Program

March 5th – 6th **Travel to Houston, Texas USA**

March 7th - 9th Houston Livestock Show, Houston, Texas.

Shep Batson
Rocking B Cattle
Wiggins, MS 39577

Jim Williams
V8 Ranch
Boling, Texas 77420

Josefina Lecuna
Santa Elena Ranch
Madisonville, Texas 77864

Bulls Eye Ranch
Brahman breeders

Ultimate Genetics
Wheelock, Texas 77882

Synergy IVF Lab
Houston, Texas

March 10th – 26th Dr Peter Hansen
Dairy Research Unit
University of Florida
Gainesville, Florida.

Dr Jim Moss
IVF Research Lab
Dairy Science Unit
University Of Florida
Gainesville, Florida.

Dr Jeremy Block
Embryologist
Embogen
Gainesville, Florida 32608

March 27th – April 04th

Andres Sobalvarro Ruata
Director
Cabanha Libre
Campo Grande, Mato Grosso do Sul.

Embrios do Sul
Campo Grande
Mato Grosso do Sul.

Yeda Fumie Watanabe
Vitrogen
Campo Grande
Mato Grosso do Sul

Dr Carlos Zanenga
Embriza
Campo Grande
Mato Grosso do Sul

Dr Pietro Baruselli
University of Sao Paulo
Sao Paulo

April 5th

Travel to Brisbane Australia

Details of the Fellowship

Background

Until recently Australia was the world's largest exporter of beef having just been overtaken by Brazil. Brazil currently produces one third of the total beef exported throughout the world. Brazil's rise to prominence in the global beef market has been rapid and the result increased productivity across many sectors of the supply chain. An area that the Brazilian beef industry has focused its resources on has been the research and implementation of Advanced Assisted Reproductive Technologies (AART). The main reproductive technology (AART) that Brazil is adopting and pioneering is that of In-Vitro Production (IVP) of embryos. In 2004, less than one half of total cattle embryo production in Brazil was from IVP. Now in 2008, IVP accounts for 100% of embryo production. This contrasts with countries like Australia where IVP accounts for less than 1% of total embryo production.

The Churchill Fellowship was planned to investigate Advanced Assisted Reproductive Technologies (AART), especially In vitro Production of cattle embryos. In particular I would be looking for aspects of this technology that could be implemented immediately into the Australian beef industry.

IVP Technology

In vitro production (IVP) of embryos involves the collection of oocytes (female gametes) from the ovary of a healthy female bovine, commonly referred to as a donor. These oocytes are collected using an ultrasound guided probe placed in the vagina of the cow. A needle penetrates the ovary and a vacuum pump draws the oocytes into a collection jar. The collection area is often located on a particular breeders' farm or ranch.

The collected oocytes are then matured for 24 hours in a 5% CO₂ incubator at 38.5 degrees C. They are then fertilized by appropriately prepared, thawed, frozen semen. This is generally done at a laboratory located away from the collection area.. Following an 18 hour fertilization period, the presumptive zygotes are transferred to a culture medium. On day 3 the presumptive zygotes are examined for the percentage undergoing cleavage. Cleavage rates of approximately 85 - 90% are quite normal. The newly formed embryos are kept in culture medium for a total of 7 days. The incubator is set at 5% CO₂ and 38.5 degrees but also contains a low oxygen tension. Trial data from the University of Florida Dairy Research Laboratory has shown significantly improved results with a low oxygen tension.

On day 7 the newly formed embryos are examined for the percentage that have formed completely into blastocysts – referred to as blastocyst rate. Commonly blastocyst rates of approximately 40% are achieved. As well, on day 7, the fully formed blastocysts are transferred into the reproductive tract of a suitable cycled recipient female. Pregnancy rates of approximately 40% can be expected though this may range from 10 – 60%.

University Of Florida IVP Protocol

I specifically went to the University of Florida Dairy Research Unit to gain access to their IVP protocol. I worked with this regime for over 2 weeks and gained valuable hands on experience. This protocol is reproduced with the permission of Dr Peter Hansen.



Dairy Research Unit staff, University of Florida with Dr Peter Hansen lower right.

Maturation of COCs

COCs (Cumulus Oocyte Complexes) collected from the ovary of a cow via ultrasound guided probe are then washed 4 times in OCM in a petri dish. The petri dish is pre-warmed on a warm plate and the microscope should be fitted with a warm stage. After the final rinse in OCM the COCs are deposited into a 50ul microdrop of OMM covered in oil. The COC's are matured for 18 – 24 hours in 5% CO₂ and 38.5 degrees Celsius.

Fertilisation Preparation

Matured oocytes are moved from the OMM plate and placed in an X plate containing HEPES TALP. The mature oocytes are washed 3 times and then transferred to a 4 well plate containing IVP TALP. These plates are then returned to the incubator.

Sperm preparation. 1 – 3 straws of frozen semen are thawed at 35 degrees Celsius for 30 seconds. This semen is layered on top of the Percoll gradient. The Percoll tube is placed

in a warmed centrifuge canister and centrifuged for 10 minutes at 200 * g. The supernatant is pipetted off and IVF TALP added to the sperm pellet.

Fertilisation

25ul of sperm/IVF TALP is added to the wells containing the matured oocytes. A further 25ul of stock 9 PHE is added to the wells. Each of the 4 well plates are returned to the incubator and fertilization is allowed to proceed for 8 – 10 hours.

Culture

The presumptive embryos are transferred from the fertilization drop to a microcentrifuge tube. The tube is vortexed for 5 minutes. The contents of the microcentrifuge tubes is then transferred to an X plate with a Pasteur pipette and searched for cumulus free zygotes. These presumptive zygotes are then rinsed in HEPES TALP twice and transferred to pre-prepared 50 ul KSOM microdrops under oil. Culture continues for a further 7 days.

Stock Solutions

- Stock 1 Na Lactate 98% Syrup
- Stock 2 Na pyruvate. Dissolve 0.22g in 100ml water.
- Stock 3 Bovine Steer Serum (BSS) Prepare 10 ml aliquots
- Stock 4 BSS/Hep Add 1000 Units sterile heparin into 500 ml BSS
- Stock 5 Oestradiol Dissolve oestradiol in ethanol to 1mg/ml
- Stock 6 Folltropin
- Stock 7 Heparin Dissolve 20mg in 10 ml water
- Stock 8 Gentamicin Dilute to 5 mg/ml and filter
- Stock 9 PHE 1nM hypotaurine, 2nm penicillamine and 250uM epinephrine
- Stock 10 Glutamine 1.5g glutamine 100ml water
- Stock 11 Glutamine 4ml aliquot
- Stock 12 MgCl₂ Mix 0.203 MgCl₂ to 10 ml water
- Stock 13 CaCl₂ Mix 0.735g CaCl₂ + 2H₂O to 5ml water
- Stock 14 Hyaluronidase 100 000 IU per 10 mls water
- Stock 15 Pen/Strep 4 ml aliquots
- Stock 16 Pen/Strep 10 ml aliquots
- Stock 24 FCS Foetal Calf Serum 100ul aliquots

Media

- Oocyte Collection Media (OCM)

Dissolve TCM 199 powder and 3.50g NaHCO₃ in 9 litres dH₂O. Adjust pH to 7.2 – 7.4 and bring volume to 10 litres.

- Oocyte Maturation Media (OMM)

87 ml aliquot TCM 199

1 aliquot stock 3 BSS

aliquot stock 8 gentamicin

125 aliquot of stock 6 Folltropin

200ul stock 5 Oestradiol

1ml stock 2 Na pyruvate

1 ml stock 10 glutamine

- Hepes TALP (Tyrodes Albumin Lactate Pyruvate) Media

500 ml TL

1.5g BSA Fract V

5ml stock 2 Na pyruvate

750 ul stock 8 gentamicin

- IVF TALP

50ml TL

300mg BSA EFAF

0.5ml stock 2 Na pyruvate

50ul of stock 8 gentamicin

250 ul stock 7 heparin

- KSOM (Potassium Simplex Optimised Medium)

Add 1ml Pen/strep to one bottle KSOM

To 5 ml KSOM add 15mg EFAF BSA, Gentamicin 2.5ul and 25ul Non Essential amino acids

Lessons from Brazil



“Cabanha Libre”, Campo Grande, Mato Grosso do Sul, Brazil.

Campo Grande was my base while in Brazil and was chosen as there were a number of functioning IVF laboratories to visit. The cattle embryo business has changed significantly in Brazil recently. Previously embryos, were “harvested” by flushing donors, 7 days after artificial insemination and superovulation. This procedure was labour intensive and required the use of numerous and repeated drug treatments of the donor animal. Today, IVF has taken over from conventional embryo transfer. It was the opinion of the laboratory staff I spoke to, that this change of technology would similarly occur in Australia.

While in Campo Grande I visited a ranch called Cabanha Libre, which was producing exceptional results from the IVP technology. While average pregnancy rates were in the vicinity of 40% and could languish as low as 20%, Cabanha Libre was producing pregnancy rates of 60% routinely. The improved pregnancy rate can be attributed to attention to detail in preparation of the recipients and donors. Specifically the breeders were using more mature heifers that were vaccinated for a range of diseases including pestivirus, black leg black disease, pulpy kidney, tetanus, botulism and leptospirosis. Overall, the ranch was concentrating on having a disease free, well developed, home grown recipient for transfer.



(L – R) Dr Guilherme Lemos, Embriza instructing Brad Pullen

Advantages of the Technology

AART offers a number of advantages over conventional reproductive techniques. Conventionally, one genetically superior cow mated with one genetically superior bull produces one genetically superior calf per year. With embryo transfer, one superior bull and cow may produce approximately 20 superior calves per year. Using the AART of In Vitro Production of embryos, a superior bull and cow may produce 200 calves per year. This would allow for the rapid spread of superior genetics throughout the particular population. This was made evident at a presentation by Dr Peter Hansen at a University of Florida field day.

A further application of the IVP technology is in obtaining genetics from younger superior females thus decreasing the generation interval. This has the effect of speeding up the rate of genetic gain, and enhancing that countries competitiveness in the global beef market. Heifers as young as 6 months were able to provide genetics to produce offspring.

During a conventional embryo transfer program, the donor is placed up the crush approximately 14 times prior to collection of the embryos. Using IVP, the donor is placed in the crush once only. This leads to a significant time and labour saving for the stud breeder. Similarly the donor receives numerous hormone injections with a conventional embryo transfer program. Under an IVP program no hormones or injections are used on the donor. This is a further cost saving to the stud breeder.

Conclusions

The Australian beef industry operates in a competitive global market place. To maintain its competitiveness in this environment the beef industry would be wise to make use of all available useful technology.

The in vitro production of embryos (IVP) is one such technology under-utilised in Australia.

In vitro production (IVP) of bovine embryos is a well recognized and applicable technology. There are well researched protocols that are delivering acceptable commercial results for the cattle industry in Brazil and the U.S.A..

There is little doubt that results achieved in the Americas can also be achieved in Australia and deliver the associated benefits. These benefits include a rapid spread of desirable genetics through a population, decreasing the generation interval and increasing the rate of genetic gain.

Care during selection of donors must be exercised so that only desirable genetics are disseminated rapidly. Astute breeding programs incorporating computer modeling eg BreedPlan, will be required to assist in the selection of appropriate female donors and sires

Recommendations

1. Establishment of IVP laboratories that cater for the production and transfer of cattle embryos in a similar fashion to those facilities that have flourished in Brazil. As of the time of writing I am in the process of building one such laboratory.
2. Dissemination of information regarding IVP to cattle producers so that they are made aware that this technology exists and is useful. In this regard I plan to hold field days and produce articles for printing in newspapers advocating, firstly the Churchill Fellowship and secondly the benefits of IVP.
3. The beef industry, government and universities might invest in the production of a suitable tertiary course that produces graduates capable of implementing commercial IVP programs as well as research into this technology to further refine its usefulness under Australian conditions.
4. Refine and develop current selection tools as well consider any overseas programs that would allow breeders to make full use of this reproductive technology.