

ARI
Final Report

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B. Reporting Period: 7/05—7/08

C. Project Number: 49627

D. Project Title: **Application of multiple marker, pre-implantation genetic testing of bovine embryos**

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J. Executive Summary:

Summarize the details of the research project.

In order to ensure a consistent supply of safe, affordable and high-quality animal based food products, producers of domestic livestock strive to improve genetic influences in their herds. Increasing the genetic potential of sires has taken place for more than 50 years through artificial insemination. With high-performing females, the collection of embryos gives producers the potential to increase selection pressure. However, the most significant potential gains will be achieved through genetic testing. Successful efforts to combine the reproductive biotechnologies with genomic techniques enable scientists to identify embryos carrying increased genetic potential for certain traits. Recent advances in gene amplification enable investigators to use sex-specific probes to determine sex in only 1 cell removed from embryos. The biopsy method has had increasing success in fresh and frozen embryos. The limiting factor on performing multiple DNA tests on an embryo biopsy is the very limited amount of DNA available in each biopsy. Recently, whole genome amplification has become a viable method of consistently and uniformly increasing the amount of DNA available from each biopsy. The proposed research will validate the MDA method for more than 2 DNA tests, and develop a strategy for producing “designer” bulls for a California producer using a combination of embryo transfer and DNA testing. Finally, a third single nucleotide polymorphism (SNP) has been discovered for μ -calpain (a tenderness gene). Briefly, cows with desirable genetics will be purchased by a coastal rancher. The cows will undergo embryo transfer at Cal Poly, the embryos will be biopsied, DNA from embryo biopsies will be amplified using polymerase chain reaction (PCR) and presence of alleles for the multiple traits will be identified. Recipient cows will be implanted with genetically tested embryos determined to have the desirable traits, i.e. bull calf with desirable μ -calpain genotype. The first objective of this study was to develop laboratory protocols for the testing for more than one gene from a single biopsy. The second objective was to apply the preimplantation genetic testing technology to a California beef herd.

K. Major Accomplishments:

State the situation of the research and the findings. Describe how the research was beneficial as well as the solutions that were found and by what means. Identify these points by numbers and/or bullets.

1. Embryos were flushed from donor cows and biopsied to test for sex and U-calpain. After biopsy some embryos were transferred fresh and others were frozen.
2. Embryos were successfully tested and transferred back to recipients with a pregnancy rate of 60%.
3. DNA from the single cell biopsies was amplified using the multi-strand displacement assay (MDA). This protocol allowed us to take a single set of DNA and make thousands of copies for multiple DNA tests to be run. 100% of the biopsies had MDA product.
4. Aliquots were taken from the MDA product to test for sex and u-calpain.
5. Multiple protocols were tested to obtain the best results for sex and u-calpain.
6. Q-PCR training was given to students and staff to aid in testing for u-calpain.
7. Calves were born with sex identified and confirmed and returned to California beef herds.

L. Impact Statements:

List the project impacts in priority order using as many numbers as needed. Please write in quotable statements or short paragraphs and be sure to include the significant accomplishments of your research and how they are likely to impact California agriculture.

As is the nature of research, these impacts may not be the ones envisioned in your original proposal.

This research grant promoted a line of research that continues to dovetail reproductive biotechnology techniques with new findings in genomics. This project clearly built upon the success of previous research in the laboratory, and most importantly, applied current technology to increase productivity of our agricultural resources.

The research described is where the industry is headed in genetic selection. Once the protocols for multiple-marker testing in embryo biopsies have been developed, producers will quickly realize that they can make a 3-year head start in selecting for traits of interest compared with traditional genetic selection methods. It is easy to envision producers collecting embryos from their very top cows, and categorizing the embryos based on the genetic traits they carry. The value of the embryos for the different categories will be such that some of the embryos from a flush will not be transferred because they carry traits that should not be propagated.

Immediate economic impact on the cattle industry is difficult to project because incorporation of the technology into practice will be gradual. However, the potential impact can be easily demonstrated by the following example: If, through testing for a genetic marker for marbling, the percentage of USDA Choice cattle can be improved from 40% to 50%, assuming a \$12 premium for Choice cattle, the return to the California beef industry would be \$6.3 million, annually. Similar calculations could be easily conducted on any number of traits.

Cal Poly was in a unique situation to perform this research. The research took steps toward developing successful protocols for multiple marker testing. Successful demonstration of the proposed technology will merge with the current embryo transfer program, and provide a service

that is not available anywhere else in the country. The project will easily evolve into a sustainable business in which Cal Poly provides the animal resources, and the genetic testing of embryos.

1. Producers were able to improve the genetics of their herd by having only embryos of the desired sex transferred to their recipients.
2. This research project validated the MDA method for 2 DNA tests, and developed a strategy for producing “designer” bulls for a California producer using a combination of embryo transfer and DNA testing.
3. Recipient cows were implanted with genetically tested embryos determined to have the desirable traits, i.e. bull calf with desirable μ -calpain genotype.

M. Dissemination, publications and presentations of research:

1. Presentation at California Agricultural Teachers Conference June 2006 and June 2007.
Given by Debbie Beckett
2. Presentation at Cal Poly Bull Test October 2005 and October 2006. Given by Jon Beckett
3. Poster presentation at Poly Royal April 2006, April 2007 and April 2008. Given by Diana Houg.
4. Manuscripts were not prepared for journals or magazines due to the fact that the PI separated from Cal Poly in June of 2007 and the statistical analysis were lacking due to low total numbers available.
5. Senior project prepared by Diana Houg. June 2007