

**ARI**  
**Final Report**

- A. Date: 2-26-06
- B. Reporting Period: 7-1-02 thru 6-30-05
- C. Project Number: 45300
- D. Project Title: “Development of Successful Sex Determination Method of Bovine Embryos Utilizing Embryo Biopsy and PCR”
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- I. Executive Summary:  
Summarize the details of the research project.

Producers of domestic livestock strive to improve genetic influences in their herds. This requires identification, and propagation of animals that demonstrate desirable characteristics. The more animals available from which to select, the greater the opportunity to discover high-performance animals. Predetermination of the sex of offspring would provide a greater number of males or females from which to select the top individuals that will contribute the genetics to the next generation. Many attempts at sexing semen and identification of sex in preimplantation embryos have been mildly successful. However, 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 variable success in fresh embryos. However, manipulation of cryopreserved embryos reduces viability of the embryos (Bredbacka, 1998). Therefore, novel approaches to improve pregnancy rates may result in effective reproductive rates. One such approach is to vary the number of manipulated, cryopreserved embryos transferred into each recipient to increase the chances of successful pregnancy. The research performed identified the most successful techniques to biopsy and sex embryos using the mouse as a model animal. DNA from collected cells was amplified using polymerase chain reaction (PCR) or sex specific probes to determine sex. Secondly, the most effective techniques were applied to cattle embryos and survival rates in micromanipulated, cryopreserved embryos were determined. Finally, recipients were implanted with one or two embryos that sex had been previously determined. Pregnancy rates, including the incidence of twinning, were recorded. The hypothesis tested was: a single method for sexing bovine cryopreserved embryos can yield high accuracy and high pregnancy rates for the desired sex.

#### Phase I – Preliminary testing

- A) PCR testing: Sarah Perkins used this portion of the grant as her Master's research project. She tested three different primers for sexing using embryo biopsy samples. Those primers included AML, FBNY, and ZFX/ZFY. All of the primers had very inconsistent results and none of them were reliable more than 50% of the time. A compounding problem was the amount of time it took to run PCR and obtain results. The embryos needed to be transferred back to the cow as soon as possible after thaw and biopsy. The minimum amount of time we could obtain results using PCR was approximately 9 hours. This was not acceptable.
- B) Ampli-Y: Catherine Mi used this portion of the grant as her senior project. She used replicate biopsies from the same embryos to validate results using the Ampli-Y test kit. She tested 156 biopsies with 152 of these matching correctly to identical blastomeres. The four incorrect tests were false females. This was attributed to the fact that the biopsy did not make it into the tube and therefore, shows a female result. This was the one downfall with the Ampli-Y testing; there was no internal control to show that DNA was in the tube. So, if the biopsy did not make it into the tube or the cell did not contain a nucleus, the result would show female. Catherine, therefore, perfected a technique to observe the biopsy enter the tube to ensure the cell was present for testing. The testing time was acceptable at 3 hours total. Ampli-Y is the sexing method that was used for the cattle in the second phase of the project, due to the more reliable results and decreased time to obtain results.
- C) FISH: The FISH procedures were not tested due to the fact that the budget was reduced when it was granted. The fluorescence attachment was unable to be purchased.

#### Phase II - Application to cattle

Fifty-two frozen-thawed embryos were biopsied and transferred to 27 recipients. The pregnancy rate was 56% (15/27) which was comparable to fresh, unbiopsied embryos. Sixteen calves were delivered (one set of twins). Sexing success rate was 75% (12/16). Of the four misdiagnosed calves one was born female that tested male and three were born male that tested female. The three misdiagnosed males could have been due to the fact that DNA was not available in the tube (the biopsied cell may not have contained a nucleus). If the cell did not

contain a nucleus and, therefore, no DNA, it would appear as a female result. However, the misdiagnosed female calf may have been due to contamination during the reaction or improper record keeping at the time of ET. Procedures have been developed to double check all steps so that the record keeping is not a problem in the future.

It was concluded that Cal Poly researchers were able to maintain a very high pregnancy rate (56%) despite the freezing and biopsy of the embryos. Accuracy of the sexing protocol was acceptable at 75% but not desirable. The fact that there was no internal control to ensure DNA was available in the tube was a major downfall. This led us to our next ARI grant where we not only amplified the whole genome but also tested for more than one trait using primers that have since been developed and are more accurate than previously tested primers. Also, biopsying before the embryos were frozen and then freezing them alleviated the tight time constraints thereby allowing us ample time to perform PCR.

J. Major Accomplishments:

1. Developed a method to effectively maintain frozen-thawed embryo viability after biopsy.
2. Successfully extracted DNA from a single cell biopsy
3. Sexing protocols were tested and identified pros and cons to each method.
4. Achieved a 56% pregnancy rate which is above the average success rate of fresh, unbiopsied embryos.

K. Impact Statements:

1. We were able to apply what we learned in further research and produce males or females for producers that desired a specific sex.
2. Clearly, the technique to biopsy does not compromise embryo viability and yields a quantity of DNA sufficient to conduct several marker tests thereby allowing us to characterize and select embryos by their genotype prior to implantation.

L. Dissemination, publications and presentations of research:

1. Fact sheet is formulated and ready to be uploaded to the CAGR website.
2. Article Published in California Cattlemen's magazine in Oct '05
3. Poster outlining ARI research permanently mounted in building 10 hall outside the biotech lab (Oct '04)
4. Presentation made at Cal Poly Bull Test Field Day by Jon Beckett (Oct '04)
5. Senior project for Catherine Mi submitted June '04
6. Presentation made at California Ag Teachers Conference by Debbie Beckett (June '03)
7. Master's Thesis for Sarah Perkins not completed yet.