Repro-BLOC™

an immunocontraceptive vaccine for population control in mammals

Amplicon Vaccine LLC
Pullman, Washington USA
January 2007
NON-CONFIDENTIAL
Presentation Contents

- Historical Information about GnRH (LHRH) Researchers
- *Repro-Bloc* is the trademark name for the GnRH (LHRH) vaccine.
- Patents, Intellectual Property and Amplicon Express Intentions
- Basic Description of GnRH Function and Fusion Protein Construct
- Data from Pilot Study performed on Heifers in the U.S.
- Data from Two Larger Scale Bull Studies Performed in Brazil
- Conclusions
- Acknowledgments
Dr. Andrew Schally, Dr. Roger Guillemin and Dr. Jerry Reeves

- After a 21 year race, Dr. Schally and Dr. Guillemin were awarded The Nobel Prize for Medicine in 1977.

- They were awarded the Nobel Prize for identifying a variety of ‘Releasing Factors’ including LHRH (Luteinizing Hormone Releasing Hormone).

- GnRH and LHRH are exactly the same molecule. Both GnRH and LHRH are prevalent in the literature.

- As a member of Dr. Schally’s team, Dr. Reeves worked on GnRH during this time.

- Dr. Reeves’ career long vision has been to use GnRH for control of reproduction and improving meat quality in domestic animals.
Dr. Jerry Reeves (continued)

- Dr. Reeves’ team has been developing fusion protein constructs using GnRH for nearly 30 years.

- He has mentored 31 Master students and 18 PhD students, many working on GnRH and associated hormones.

- His publication record includes more than 100 peer reviewed manuscripts on GnRH and related subjects.

- He is currently at Washington State University, Department of Animal Sciences.
Patents and Intellectual Property

- U.S. Patent No. 6,013,770, “Chimeric Contraceptive Vaccines,” Jerry J. Reeves, Kevin P. Bertrand, and Yuzhi Zhang, issued January 11, 2000 (WSURF #363)
- U.S. Patent No. 6,045,799, “Chimeric Contraceptive Vaccines,” Jerry J. Reeves, Kevin P. Bertrand, and Yuzhi Zhang, issued April 4, 2000 (WSURF #363)
- U.S. Patent Application Serial No. 09/524,974, “Chimeric Contraceptive Vaccines,” Jerry J. Reeves, Kevin P. Bertrand, and Yuzhi Zhang, filed March 14, 2000 (WSURF #363; this is a continuation application)
- All Intellectual Property associated with this vaccine is exclusively licensed to Amplicon (willing to sublicense on exclusive basis).
- Available under the brand name *Repro-Bloc*
Amplicon’s Intentions for Realizing the Technology in the Marketplace

- There are a variety of market opportunities for the vaccine: heifers in USA, bulls in Brazil, deer in USA, lambs in Middle East, swine in China and feral (wild) dogs/cats in various countries and non-human primates (India).
- Utilization in food chain animals will require government approval. Amplicon has already sublicensed the technology to a firm for use in Elephants and Lions and is looking for a company capable of bringing the vaccine to market in food chain animals.
- Amplicon is in the process of obtaining US government approval for use in into ‘non-food chain’ animals (i.e feral dogs/cats).
- Completed studies which are NOT discussed in this presentation: mouse, rat, swine, dogs, cats, lamb, caribou, elephant, lion, and other cattle studies.
- Data NOT discussed in this presentation: antibody data from various studies, dosage studies, non-responder studies, and long-term mouse studies for addressing reversibility.
- Data from all these studies are available to interested firms (MCA required).
Natural Function of GnRH in Mammals

*Vaccine works in male AND female mammals*

**Hypothalamus**

**GnRH**

**Pituitary**

(GnRH releases FSH & LH)

**FSH/LH**

- **Follicular Growth, Ovulation Estrogen** (Effects Female Mammals)
- **Sperm Production Testosterone** (Effects Male Mammals)
Design of the Vaccine

- A series of GnRH genes are cloned into Ovalbumin carrier gene which is held in an E. coli based expression vector.

- This construct is expressed as a fusion protein, isolated and purified.

- The purified protein is added to an emulsifying agent, oil, dead mycobacterium butyricum and a urea + phosphate buffer.

- The vaccine is injected subcutaneous into animals to cause an immune response against GnRH.

- Antibodies to the GnRH fusion protein remove naturally occurring GnRH from the animal and prevent FSH/LH release.

- The vaccine causes sterility in both males and females.
Action of GnRH Vaccine in Mammals

Antibodies to GnRH vaccine NEUTRALIZE naturally occurring GnRH in the animal

Hypothalamus

GnRH  $\iff$  GnRH Antibody

---X---

(GnRH stops interacting with Pituitary)

Pituitary  (FSH & LH remain in the Pituitary)

PREVENTING ESTROGEN & TESTOSTERONE PRODUCTION
(causing sterility in male and female mammals)
Pilot Study: Heifers in the USA

- **Problem:** Prior to slaughter, US cattle ranchers move Heifers into expensive ‘feed lots’ to increase meat quality and quantity. Pregnant Heifers spend valuable resources on the fetus and not on meat production.

- **Hypothesis:** Heifers immunized with the GnRH vaccine will earn more money for the producers by not becoming pregnant.

- **Objective:** Prove the effectiveness of GnRH vaccine in preventing Heifers from cycling (cycling is a requirement to become pregnant).

- **US Market Size:** 12 Million Heifers per year are slaughtered.
GnRH Injection Timing and Percent Cycling Data

% Heifers Cycling

% GnRH 125 I Binding

Days

1st Injection
2nd Injection
3rd Injection
GnRH Immunized Heifers vs. Intact Heifers

Week

% Cycling

Intact n=10

GnRH n=39
Scientific Study: Heifers in the USA

• **Results:** All Heifers in the study stopped cycling after being immunized with the GnRH vaccine. Suppression of estrus is correlated to GnRH antibody titers.

• **Current Studies:** To quantify the increased meat quantity and quality from immunized Heifers.

• **Issues:** Obtaining FDA approval for the use of the GnRH vaccine (in US Heifers) will require years of clinical trials.
Scientific Study: Bulls in Brazil

- **Problem:** Intact Brazilian bulls have low quality meat and are very aggressive (fighting and damaging property). Traditional castration creates a variety of problems including infection, screw worm infestation and possible death (1% to 3%).

- **Hypothesis:** GnRH immunized bulls will have a quality of meat and carcass weights similar to traditionally castrated bulls.

- **Objective:** To determine if the GnRH vaccine is a cost effective, humane alternative to castration.

- **Brazilian Cattle Market Size:** 176 Million bulls are slaughtered per year.
Locations for Experiment: Brazilian Bull Study

• Two Ranches in Mato Grosso Region of Brazil
  – John Carter (Esperanca Ranch) n=77 animals
  – Dr. Hugo (Colorado Ranch) n=216 animals

• Esperanca Ranch animals were two years old when the study was started and were slaughtered 387 days after the initial immunization.

• Colorado Ranch animals were two years old when the study was started and were slaughtered 741 days after the initial immunization.
Methods: Brazilian Bull Study

- **Three treatment groups**
  - GnRH immunized (n=98)
  - Castrated (n=98)
  - Intact Bulls Control (n=97)

- **Measure Body Weights**

- **Measure Scrotal Circumferences**
  - Decreased circumference indicates vaccine is functioning properly

- **Measure Testes and Epididymis Weights after slaughter**
  - Decreased weight indicates vaccine is functioning properly

- **Collect and Analyze Meat Quality Data**
  - Marbling, Fat Content, Tenderness, Flavor and Juiciness
### Study Overview: Injection and Castration Dates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Orange</th>
<th>Green</th>
<th>Yellow</th>
<th>GnRH-Im</th>
<th>Castration</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GnRH-Im</td>
<td></td>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Orange</th>
<th>Green</th>
<th>Yellow</th>
<th>GnRH-Im</th>
<th>Castration</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
<th>Slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>141</th>
<th>287</th>
<th>387</th>
<th>741</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Esperanca Animals**: n=77
- **Colorado Animals**: n=216
Body Weight of Castrated, Immunized and Intact bulls
Esperanca Animals (n=77)
Body Weight of Castrated, Immunized and Intact bulls

Colorado Animals (n=216)
Scrotal Circumferences of Castrated, Immunized and Intact bulls

Esperanca Animals (n=77)

GnRH Immunizations
Scrotal Circumferences of Castrated, Immunized and Intact bulls
Colorado Animals (n=216)

GnRH Immunizations

Scrotal Circumference (cm)

Day of Study

Castrated
Immunized
Intact
Testes Weight* on Day 387
Esperança Animals (n=77)

*Lower Testes Weight indicates the vaccine is working
Testes Weight* on Day 741
Colorado Animals (n=216)

*Lower Testes Weight indicates the vaccine is working
Epididymis Weight* on Day 387
Esperanca Animals (n=77)

*Lower Epididymis Weight indicates the vaccine is working
Epididymis Weight* on Day 741
Colorado Animals (n=216)

*Lower Epididymis Weight indicates the vaccine is working
Meat Quality Study Methods

- Various cuts of meat were analyzed from 30 animals of the three treatment groups
  - GnRH immunized (n=10)
  - Castrated (n=10)
  - Intact Bulls Control (n=10)

- All meat grading and subjective evaluations were performed by an independent panel of meat experts.

- These Meat Quality data were recently submitted to “Meat Science” (a peer reviewed journal). The manuscript is included on the CD-ROM.
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

**Marbling**

*A larger number equates to increased marbling and higher meat quality.

*A larger number equates to increased marbling and higher meat quality.*
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

*Percentage of Carcass Fat

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Castrated</th>
<th>Immunized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>17.9</td>
<td>23.9</td>
<td>25.2</td>
</tr>
</tbody>
</table>

*A larger number equates to increased fat and higher meat quality.*
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperança Animals Only (n=30)

*Fat Thickness, mm

*Thicker fat in the region measured indicates higher quality meat.
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

*Tenderness (Taste Panel)*

- Intact: 5.0
- Castrated: 5.5
- Immunized: 5.3

* A blind, subjective evaluation by a panel of meat tasting experts, larger number is higher meat quality.
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

*Tenderness (Texturometer in Newtons)

*A smaller number equates to increased tenderness and higher meat quality.
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

*Meat Juiciness

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Castrated</th>
<th>Immunized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meast Juiciness</td>
<td>6.7</td>
<td>6.9</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Variation from 1 to 9, where 1 = meat extremely dry, undesirable flavor, tough; 5 = average; 9 = meat extremely juicy, flavorful and tender.
Meat Quality Data of Intact, Castrated, and GnRH immunocastrated Esperanca Animals Only (n=30)

*Meat Flavor

*Variation from 1 to 9, where 1 = meat extremely dry, undesirable flavor, tough; 5 = average; 9 = meat extremely juicy, flavorful and tender.
Meat Quality Data Conclusions

• Beef from immunized animals has preferable better fat content and marbling structure (when compared with intact animals).

• Beef from immunized animals is comparable in tenderness, juiciness and flavor to that of castrated animals (and far superior to intact animals).

• Weaknesses: only 30 animals were evaluated in this study.
Overall Study Conclusions

- The price of BULL meat in Brazil is 37 *REAL/15kg.
- The price of STEER (immunized) meat in Brazil is 41 REAL/15kg.
- Based on the results of these studies, a rancher injecting his animals with the GnRH vaccine would make 20.30 REAL more per animal opposed to leaving the animals as intact bulls**.
- For ranchers using traditional castration procedures: if ONLY one animal dies from castration complications, then the rancher makes an additional 704.05 REAL by using the vaccine (assuming a 257.6 Kg carcass).
- We think Brazilian Cattle Ranchers would prefer the GnRH vaccine to traditional castration practices or raising intact bulls.
- Note- obtaining Brazilian government approval for the use of this vaccine in Bulls, will probably require less time and money than obtaining approval in the US Heifer market.

*One Brazilian REAL is equal to 0.35 US DOLLARS (on 10/13/2003)

**In Brazil the usable carcass is 53% of the animal weight- assuming intact bulls 523 Kg & GnRH immunized 486 Kg.
Acknowledgments

- Jerry J. Reeves: Washington State University
- Jennifer Hernandez: Washington State University
- David DeAvila: Washington State University
- Kevin Bertrand: Washington State University
- Eraldo Zanella: Universidade de Passo Fundo
- Edson L. de A. Ribeiro: Universidade Estadual de Londrina
- Heather Marney: Amplicon Vaccine LLC
- Keith Stormo: Amplicon Vaccine LLC

For additional information contact:
  Robert Bogden
  President
  Amplicon Vaccine LLC
  2345 NE Hopkins Court
  Pullman, Washington 99163 USA
  1-509-332-8080 ext. 24
  <bogden@genomex.com>