**BIOENGINEERING OF THE MAMMARY GLAND OF LIVESTOCK: INCREASED PROPEPTIDE PROCESSING OF FACTOR IX IN THE MILK OF TRANSGENIC PIGS BY CO-EXPRESSION OF FURIN**

Jianguo Zhao1,2, Eric M. Walters1, J. Calcaterra3, Jason W. Ross2, Lee D. Spate2, Melissa S. Samuel1, August Rieke2, Clifton N. Murphy2, Steven Butler2, William H. Velander3, Randall S. Prather1,2

1 National Swine Resource and Research Center, University of Missouri-Columbia MO, USA
2 Division of Animal Sciences, University of Missouri-Columbia, MO, USA
3 Department of Chemical and Biomolecular Engineering, Lincoln, NE, USA
4 Department of Dairy Science, Blacksburg, VA, USA

---

**Introduction**

**Hemophilia**
- Hemophilia is a rare inherited blood clotting disorder.
- Affects 18,000 people in the U.S. with 400 new cases/year.
- Hemophilia usually occurs in males (X-linked recessive inheritance).
- **Complications:**
  - Prolonged bleeding after an injury or accident.
  - Bleeding in the joints causes pain / arthritis.
  - Bleeding in the brain - requires emergency treatment

**Common cause:** congenital deficiency in clotting proteins:
- Factor VIII (Type A) 1:5000 males
- Factor IX (Type B) 1:23,000 males

**Rationale**
- The milk of 60 transgenic pigs (12,000 liters/year) could supply the entire amount of Factor IX needed in the United States for hemophilia B patients

**Objective**
- To produce transgenic pigs expressing recombinant human Factor IX as a safe, abundant, and inexpensive source of the protein for the biomedical community.

**Specific aim**
- Production of triple transgenic pigs as mammary bioreactors for:
  - Factor IX
  - Furin (PACE: Paired basic Amino acid Cleaving Enzyme)
  - Alpha 1-antitrypsin (AAT; SERPINA1)

**Conclusion**
- 3 females and 15 male tri-transgenic FIX founder pigs were developed by SCNT.
- Furin expression increased propeptide cleavage, with complete processing of pro-FIX to FIX.
- Alpha-1-antitrypsin was co-expressed and did not inhibit furin processing of the pro-FIX.
- We observed significant expression of hFIX in the mammary gland of transgenic pigs with increased propeptide processing of FIX by co-expression of furin.

---

**Methods**

**Results**

**SCNT Founders:**
- # Clones
  - 3 females
  - 10 males

**Tri-transgene transmission offspring**
- F0 n = 4
- Tri-transgenic
- Wild-type

**PCR genotyping results:**

**Transgenic milk proteins from induced lactation in F0 SCNT females**
- F0 female #1 – Factor IX was expressed, but not AAT.
- F0 female #2 – Factor IX was expressed, and AAT was detected.

**Conclusions**

**Support**
- National Center for Research Resources RR18877 and R01 HL078944.