I prefer:

☑ ORAL presentation

□ POSTER presentation

**A single-chain variable fragment (scFv) antibody against plasma membrane epitopes of Y- sperm boar: challenging for sexing bull and buffalo sperm**

Marninphan Thongkham a,1\*, Korawan Sringarm1, Supamit Meychay1, Surat Hongsibsong2, Anucha Sathanawongs3

\*lead presenter

1 Marninphan\_t@cmu.ac.th, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand

2 School of Health Sciences Research, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand

3 Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

**Abstract:**

**Background/Objective:** The livestock industry prefers sperm sexing techniques. The immunological sexing approach is an alternate method for applying sexing sperm. A single-chain variable fragment antibody against plasma membrane epitopes of the Y-sperm boar (Boar-scFv) was produced, and it had high efficacy when used to separate X- and Y- boar sperm. This achievement holds intriguing potential applied for sexing sperm from bulls and buffaloes. Thus, this research focuses on the efficiency of boar-scFv for the sexing of bull and buffalo sperm.

**Methods:** Semen samples were taken from three tropical Holstein Friesian bulls (*Bos taurus*) and three swamp buffalo bulls (*Bubalus bubalis)* aged 3–5 years. Magnetic-activated cell sorting combined with Boar-scFv was applied for sexing sperm. Boar-scFv (0, 0.5, 1, and 2 mg/mL) was conjugated with PLA-M magnetic microbeads (BS-beads). Each semen from bulls and buffaloes was diluted in a Tris–citric acid-based extender to 4 × 106 cells/mL and incubation with BS-beads. After incubation, the sperm sample were separated by unbonded (supernatant fraction) and bonded (eluted fraction) with BS-beads. Then, semen samples were determining sex ratio by imaging flow cytometry.

**Results:** Efficient sexing of buffalo and bull sperm was indicated by BS-beads. The optimal concentration of Boar-scFv antibody coupling to the surface of magnetic microbeads for sexing bulls and buffalo sperm was 1 mg/mL. BS-beads were revealed high significantly percentage enriched Y-sperm in the eluted fraction (Bull 65.1 – 67.2 %, Buffalo 68.4 – 71.3%) and showed high significantly percentage X-sperm in supernatant fraction (Bull 63.4 – 66.7 %, Buffalo 65.3 – 69.2%) when compare with conventional semen.

**Conclusion:** Bulls and buffalo sperm sexing with BS-beads demonstrated high efficiency in separating Y-sperm and X-sperm. This initial technique for Boar-scFv involves sexing bulls and buffalo sperm.

**Keywords:** Sexing semen, scFv antibody, Boar, Bull, Buffalo