



**SOUTH DAKOTA  
STATE UNIVERSITY**

Department of Animal Science

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## Reproduction

### Use of Sperm Proteins as a Putative Fertility Marker

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#### Objective

The objectives of this study were to characterize the variation and evaluate whether CD9 and SERPINA5 could be used as fertility markers in bovine sperm.

#### Study Description

Semen from 17 bulls of three different breeds (Angus, Simmental and Hereford) were evaluated for presence of CD9 and SERPINA5 (study 1). Then, two separate ejaculates (mean 5 months apart) from 23 bulls were used to evaluate CD9 and SERPINA5 concentrations on the sperm head (study 2). Bulls (study 2) were classified based on sire conception rates (SCR) as HIGH (n = 12) or LOW (n = 11) fertility (minimum of 6.5 SCR units difference with an average of 13,443 inseminations per sire). Frozen semen was thawed, and an aliquot stained with Hoechst 33342 and propidium iodide for analyses of total and progressive motility, and viability. The remaining semen was fixed with formaldehyde, and incubated with antibodies for either CD9 or SERPINA5. Samples were analyzed for fluorescence intensity (FI) on a minimum of 100 sperm heads per sample. Statistical procedures were performed in SAS using the GLM in study 1 to evaluate if the variance in protein was greater between bull compared to within bull and in study 2 the GLIMMIX (total and progressive motility, and viability) and MIXED (fluorescence intensity) procedures were used. The CORR procedure (study 2) was used between SCR, CD9 and SERPINA5. All samples (study 1) passed quality control for frozen-thawed semen and were from a CSS certified AI stud (total and progressive post-thaw motility were  $32.6 \pm 12.5\%$  and  $19.0 \pm 8.3\%$ , respectively).

#### Take home points

Both CD9 and SERPINA5 were localized to the sperm head. However, SERPINA5 was also detected on the proximal region of the sperm tail among all bulls ( $33.5 \pm 4.1\%$  of sperm; range 4% to 61%). Variation in FI on the sperm head was greater for both CD9 ( $P < 0.001$ ; FI range  $14.1 \pm 0.17$  to  $19.9 \pm 0.18$ ) and SERPINA5 ( $P < 0.001$ ; FI range  $12.9 \pm 0.35$  to  $19.0 \pm 0.36$ ) among bulls (variance of 5.96 and 15.14, respectively) compared to within bulls (variance of 3.32 and 13.03, respectively). In summary, results from study 1 demonstrated that CD9 and SERPINA5 are potential fertility markers. In study 2, there were no differences ( $P > 0.30$ ) in total and progressive motility between high and low fertility and no ejaculate by fertility interaction. However, there were differences in both total and progressive motility between ejaculates ( $P < 0.01$ ). There were no differences in viability ( $P > 0.20$ ) between ejaculates and no ejaculate by

fertility interaction; however, HIGH had greater viability compared to LOW ( $P < 0.05$ ;  $64.0 \pm 2.4$  vs.  $56.9 \pm 2.3$ ). Neither CD9 nor SERPINA5 were correlated with SCR ( $P > 0.30$ ); however, CD9 and SERPINA5 were correlated with each other ( $P < 0.01$ ;  $r = 0.53$ ). There were no differences between HIGH and LOW for either CD9 or SERPINA5 ( $P > 0.40$ ). There were differences between ejaculates ( $P < 0.01$ ;  $31.6 \pm 0.6$  vs.  $31.0 \pm 0.6$  and  $52.4 \pm 1.4$  vs.  $53.6 \pm 1.4$ , for CD9 and SERPINA5, respectively). There was also an ejaculate by fertility group interaction for SERPINA5 ( $P < 0.01$ ) with ejaculate 1 and 2 being different ( $P < 0.01$ ) among HIGH ( $50.6 \pm 1.9$  vs.  $53.3 \pm 1.9$ ) but similar ( $P > 0.20$ ) among LOW. In conclusion, CD9 and SERPINA5 changed over time; however, there was no relationship between their relative concentrations and SCR among the bulls in study 2. In summary, results from study 1 demonstrated that CD9 and SERPINA5 are potential fertility markers. Also, CD9 and SERPINA5 changed over time; however, there was no relationship between their relative concentrations and SCR among the bulls in study 2.

**Keywords:** bull, fertility, semen, sperm protein