Characterizing DNA methylation of the LGALS14 promoter in low- and high-fertility bulls and their conceptuses

María Del Carmen Velez-Colon[1,2], M. Sofía Ortega[2], Rocío Melissa Rivera[2]

[1]University of Puerto Rico-Ponce, Ponce, PR, 00732 and [2]Division of Animal Sciences University of Missouri, Columbia, MO 65211

In dairy cattle, the greatest pregnancy loss occurs around the third week after fertilization; a period where we have found downregulation of *LGLAS14* transcript in conceptuses of low fertility bulls. In humans, *LGALS14* is a placenta-specific gene that has been suggested to have a key function in the maintenance of pregnancy and its downregulation has been implicated in preeclampsia. DNA methylation, the addition of a methyl group onto the fifth carbon of the cytosine when in a CpG context, is an epigenetic modification associated with gene silencing when found in promoter regions. Therefore, we hypothesized that downregulation of *LGALS14* in conceptuses of low fertility bulls is due to DNA methylation in the promoter of this gene in the sire's sperm cells. Our objective was to determine if the proximal promoter of *LGALS14* is differentially methylated in spermatozoa of two high and two low fertility bulls and their conceptuses. For this, we isolated sperm cell DNA from the four bulls and performed bisulfite sequencing to identify DNA methylation status at five CpGs within the proximal promoter (-283 bp from the transcription start site). Preliminary results show differences in promoter methylation between bulls. During bisulfite mutagenesis, unmethylated cytosines (C) are converted to uracils and substituted with thymines (T) during PCR. Thereby, to ensure that queried cytosines are unmethylated (*i.e*. T after PCR) and not the result of single nucleotide polymorphism between bulls (*i.e*. C in one bull and T in another), genomic sequencing of the *LGALS14* proximal promoter was conducted. Next, we performed bacterial transformations with the bisulfite-converted PCR product of each bull and 2-3 of their conceptuses, in order to establish methylation patterns of individual chromosomes. We are currently awaiting the sequencing results. Our study will contribute to the identification of epigenetic markers that could influence semen quality and pregnancy establishment.