Aberrant DNA methylation patterns have been associated with the failures during animal reproduction and development. Using cattle as a model, we are studying one of the major epigenetic components: DNA methylation in various tissues, gamete production, early embryo development. We have systematically characterized the impacts of tissue types, ages and generations on the DNA methylation status in cattle. We detected over 34 million potential methylated sites of ten tissues from three cattle using RRBS method. An average of 1.5% sites were detected with methylaton level when we use >7 reads as a threshold. These results will be used for detecting the relationship between methylation level and genome structures, the relationship between methylation level and gene expression when combined with the published RNA expression data, and the methylation differences among different tissues. To estimate the impacts of DNA methylation on animal fertility, we are in the process of generating genome- wide DNA methylation maps at a single-base resolution with bull sperms of high and low fertility. These results will provide insights into the roles of DNA methylation in animal reproduction and development.

**290. Nutritional Programming of Accelerated Puberty in Heifers: Alterations in DNA Methylation in the Arcuate Nucleus.**

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High rates of body weight gain during the juvenile period appear to program molecular events within the hypothalamus, leading to advancement of puberty. The hypothalamic arcuate nucleus (ARC) integrates nutritional inputs through intermediate neuronal or glial circuits that regulate GnRH release, and differential gene transcription within the ARC appears to be involved with nutritional programming leading to accelerated puberty in heifers. This is supported by our previous studies reporting altered mRNA abundance of key genes associated with the control of GnRH release, neuronal plasticity, and signaling pathways in the ARC, and accompanying increases in adiposity and secretion of leptin and insulin-like growth factor 1 (IGF-1). Methylation of DNA, an epigenetic mechanism that controls gene expression, is associated with metabolic programming events and proposed to play a role in the pubertal process. Herein, we hypothesized that an altered pattern of DNA methylation in key genes within the ARC occurs in response to an elevated rate of body weight gain during the juvenile period. We assessed DNA methylation in the ARC of juvenile heifers fed to gain body weight at relatively high (1 kg/day; High- gain, n = 4) or low (0.5 kg/day; Low-gain, n = 4) rates from 4.5 to 8.5 mo of age. At the completion of the experiment, heifers (as anticipated) remained prepubertal, with earliest puberty expected around 9 mo of age in High-gain heifers. A block of tissue containing the hypothalamus was dissected, snap frozen, cut in 20-µm coronal sections, mounted on slides, and stored at -80$^{o}$C. Genomic DNA was isolated from bilateral tissue scrapes of a 1-mm diameter area within the ARC and a methyl-cytosine enrichment assay was used to capture methylated regions of the genome. Using a custom-designed oligonucleotide array targeted to imprinted genes and genes associated with nutritional inputs and the control of puberty, a comparative-genomic-hybridization array was used to identify differentially methylated regions between High- and Low-gain heifers. Treatment effects on DNA methylation patterns were assessed by *t*-test and by Fisher's exact test (comparing the proportion of heifers of each treatment identified as exhibiting hypermethylation of a given sequence). Differential methylation of genomic regions was observed at genes involved in the modulation of growth and metabolism, including those encoding the receptors of growth hormone (*GHR*), IGF-1 (*IGF1R*) and leptin (*LEPR*), as well as the imprinted *IGF2* and *PEG3* genes. Importantly, differential methylation was observed at *LIN28B* and *HMGA2,* genes recently proposed to be central components of a key inhibitory network that controls onset of puberty. Hence, an elevated rate of body weight gain during the juvenile period alters DNA methylation patterns in the ARC and these changes may be critical for programming the age at puberty in heifers. Collectively, these findings show dietary dependent epigenetic modulation of metabolic pathway genes within the ARC. Supported by USDA-NIFA-AFRI 2009-65203-05678.

**291. Karyotyping analysis and epigenetic characteristics of porcine ESCs.**

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Pluripotent stem cells can be categorized according to their pluripotent state. The distinct biphasic states, naïve and primed, represent cells of the preimplantation embryo and later epiblast cells, respectively. However, naïve stage in pigs has been difficult to capture *in vitro*.We already reported that primed embryonic stem cell (ESC) lines were derived from porcine embryos of various origins, including *in vitro* fertilized (IVF), parthenogenetic activation (PA) and, in particular, nuclear transfer (iPS-NT) from a donor cell with iPSC. Karyotyping analysis revealed that the representative four cell lines contained a normal number of 38 XX chromosomes at 10-25 passages. The pericentric inversion of chromosome 8, which is considered a normal variation, has been detected in IVF 0214 and PA 0531B lines. The expression of Xist has only been able to observe in female porcine ESC lines by RT-PCR, except for the male cell line TG-NT1, which was established recently. Additionally, immunofluorescence staining with H3K27me3 as a marker of the state of XCI has been performed in porcine ESC lines. Interestingly, only iPS-NT lines showed multiple aberrant patterns of nuclear foci, whereas other female cell lines had a single-positive focus and male cell lines did not have a focus in the nucleus. These observations suggest that iPS-NT lines have epigenetic instability compared with other porcine ESC lines.
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**292. Tissue-specific expression of estrogen receptor 1 is regulated by DNA methylation in a T-DMR (tissue-dependent and differentially methylated region).**

RYO MAEKAWA[1], MAKI OKADA[2], HIROSHI TAMURA[2], NORIHIRO SUGINO[2].

# SSR 2015 Annual Meeting: Evolution of Sex

**Date:**
June 18, 2015 (All day) to June 22, 2015 (All day)
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Meeting

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