Incomplete Compensatory Up-regulation of X-linked Genes in Bovine Germline, Early Embryos, and Somatic Tissues

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Abstract
The maintenance of a proper gene dosage is essential in cellular networks. To resolve the dosage imbalance between eutherian females (XX) and male (XY), X chromosome inactivation (XCI) occurs in females, while X chromosome dosage compensation (XDC) in males active the X to balance its expression with that of autosome pairs [Ohno’s hypothesis]. These phenomena have been well studied in humans and mice, despite many controversies over the existence of such X chromosome up-regulation. Using RNA sequencing data, we determined X chromosome dosage compensation in the bovine by analyzing the global expression profiles of germ cells, embryos, and somatic tissues. Our analyses showed a decreased relative X to autosomal gene (A) expression (RXE) after fertilization, indicating that the sperm that undergo meiotic sex chromosome inactivation bring in inactive X chromosomes to the matured oocytes. Subsequently, the activation of the bovine embryonic genome at the 4-8 cell stage increased RXE from −0.54 to −0.05. This was followed by a sharp RXE decline from −0.02 at the 16-cell stage, 0.1 at the 32-cell stage to −0.29 at the compact morula stage, which is known as paternal X inactivation stage in the bovine. Finally, RXE was stabilized from blastocysts (~0.19) through Day 19 conceptuses (~0.25) to the somatic tissue average (~0.21), suggesting a pattern of incomplete X compensation.

Method
Eight bovine RNA-seq datasets (Table 1), covering the bovine immature/mature oocytes, pre-implantation conceptuses (Figure 1), extra-embryonic tissues, and male/female somatic tissues, were obtained from the Gene Expression Omnibus. These datasets, representing 4 chromosome scenarios in cells, XX:XXA (diploid immature oocyte with DNA duplication), XX:XXA (haploid mature oocyte with DNA duplication), XX:A (XXA and X:A (gradual changed X status in bovine pre-implantation conceptuses), and X:A (extra-embryonic tissues and somatic cells in bovine with one active X or XY male), were analyzed for dosage compensation as shown in Figure 2. A total of 959 X-linked genes and 20,316 autosomal genes were used to calculate the relative X to autosomal gene (A) expression (RXE): log2(X expression) – log2(A expression) (Figure 3).

Results
I. Datasets distribution overview
Table 2. The numbers and percentages of paralogs (>70% similarity) as well as the total numbers of annotated genes in each bovine chromosome.

Table 3. Incomplete X chromosome dosage compensation in bovine adult somatic tissues.

Figure 1. Bovine pre-attachment embryo development.

Figure 2. RNA-seq data analysis workflow. Reads were trimmed and non-uniquely (paralogs included) mapped to the bovine reference genome assembly UMD3.1.1 using Hisat2 (version 2.0.5) aligner. The mRNA level for each gene, estimated by transformed transcripts per kilobase million (TPM) was quantified by IsoEM (version 1.1.5).

Figure 3. Formula for the calculations of the relative gene expression (RXE) for each chromosome (black dots) and relative X expression (RXE; red dot). X Chromosome dosage compensation definition and visualization.

Figure 4. Expression ranges of bovine chromosomes in representative bovine immature oocytes. a. The ranges and medians of X-linked gene expression (red; TPM > 0.3) were similar to those of autosomal pairs (blue). b. A representative empirical cumulative distribution (ECD) plot showing that the distribution of X-linked gene expression (TPM > 0) was similar to those of autosomal pairs.

Figure 5. Incomplete X chromosome dosage compensation in bovine germ cells and embryos. Boxplots show the relative gene expression values for each chromosome. Red dash lines indicate equal average expression value among all chromosomes. The black dot indicates relative gene expression for each chromosome, the red dot represent the RXE.

Conclusions
No significant RXE differences were observed between bovine female and male somatic tissues; X:A ratios in bovine germ cells, early embryos and somatic tissues were >0.5. Our data thus support X expression up-regulation as proposed by Ohno, who predicted a balance in the expression of X-linked genes to that of autosomes.

Acknowledgements