UCONN | College of Agriculture, Health and Natural Resources
Animal Science

Dosage Compensation of the X Chromosome in Ovine Embryos, Fetal and Adult Somatic Tissues

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Abstract

Deviations from proper gene dosage can cause severe to lethal consequences in mammals. Eutherian males (XY), however, have reduced gene dosage compared with females (XX) due to a single X and deteriorating Y chromosome. This dosage imbalance is resolved through X chromosome dosage compensation, according to Ohno's hypothesis: X-linked gene expression is doubled in both males and females to balance expression of the X chromosome and autosomes. To compensate for the X chromosome over expression in females, X chromosome inactivation (XCI) inactivates a single X chromosome in each cell. Although many studies have been done in mice and humans, controversies exist due to the analysis and interpretation of RNA sequencing data. Here we described the first X chromosome dosage compensation in the sheep. Control, restricted, and overfed ovine fetal somatic tissues displayed incomplete dosage compensation. Incomplete dosage compensation was also observed in juvenile and major organs of adults and female specific tissues. Brain, apart from the cerebellum, displayed complete dosage compensation with an *RXE* range of 0 to 0.16. An interesting pattern was observed in the male specific tissues with complete dosage compensation in the epididymis (*RXE* = 0.32) and incomplete dosage compensation in the testes (*RXE* = −0.84). No significant *RXE* differences were observed between ovine female and male somatic tissues, supporting Ohno's hypothesis of balanced expression of X-linked genes to autosomal genes. Our results indicate that a mechanism for dosage compensation exists in the sheep, although it is largely incomplete.

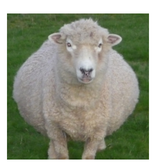
Materials and Methods

Twelve pregnant ewes were fed 100% (control), 60% (restricted), or 140% (overfed) of the National Research Council requirements for a ewe pregnant with twins (NRC, 1985; Nutrient Requirements of Sheep, 6th ed.). Brains, lungs and kidneys were collected from Day 135 fetuses of the control, restricted, and overfed groups (n = 7, 4, and 4; respectively). RNA-seq libraries were prepared using the Illumina TruSeq stranded mRNA kit and sequenced on the NextSeq 500 (Illumina Inc., San Diego, CA, USA). Two additional RNA-seq datasets were downloaded from Sequence Read Archive (SRA), including Day 14 embryos (PRJNA254105), and adult and juvenile heart, brain, liver, muscle, rumen, and female- and male-specific tissues (PRJEB6169) (**Table1**). Data were analyzed as shown in **Fig. 1**. The relative expression of X to autosomal (A)(*RXE*) was calculated using *RXE* = $\log_2$(X expression) – $\log_2$(A expression) with an average of 486 X-linked and 13,001 autosomal genes after TPM >1 filtering. RXE ≥0 (or X:A ratio ≥ 1); <0, or −1 indicate complete, incomplete, or no dosage compensation; respectively.

Pregnant ewe nutrition treatment groups

Restricted (N=4) (60% NRC)

Control (N=4) (100% NRC)



Overfed (N=4) (140% NRC)

Tissues from Day 135 fetuses: Brain, Kidney, and Lung

↓ RNA-sequencing

Table 1. Additional RNA-seq datasets used.

Tissue Type	Number of samples	Library type	BioProject ID	Reference
Heart 4 regions of brain Liver Muscle Rumen Female specific tissues Male specific tissues	N=6 N=4,4,6,8 N=4 N=4 N=8 N=4 N=2,4	Paired-end Illumina	PRJEB6169	EBI
Day 14 embryos	N=4	Single-end Illumina	PRJNA254105	Brooks et al., 2015

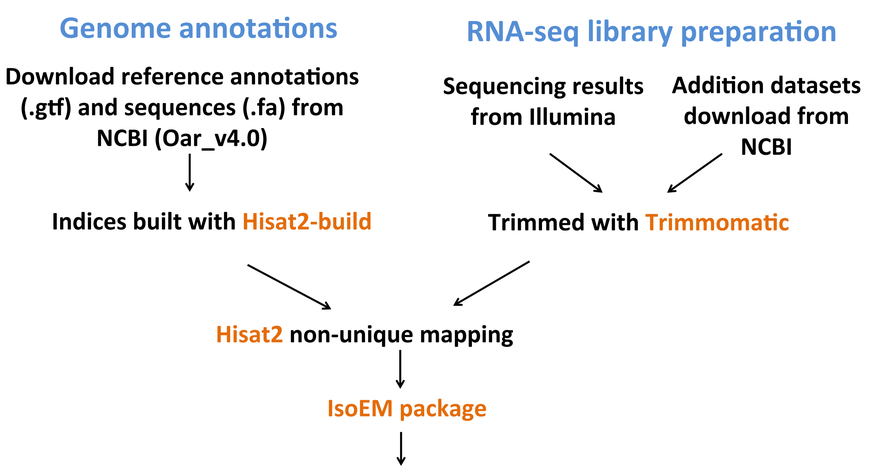


Fig. 1 RNA-seq data analysis workflow. Reads were trimmed and non-uniquely (paralogs included) mapped to the ovine reference genome assembly Oar_v4.0 using Hisat2 (version 2.0.5) aligner. The mRNA level for each gene, estimated by transformed transcripts per kilobase million (TPM) was quantified by IsoEM (version 1.1.5).

$$RGE = log_2(\frac{i}{n-i}) = log_2(i) - log_2(n-i)$$

$$RXE = log_2(\frac{x}{a}) = log_2 x - log_2 a$$

RXE > 0:	X:A ratio > 1
	Dosage Compensation
-1 < RXE < 0:	0.5< X:A ratio < 1
	Incomplete Dosage Compensation.
RXE = -1:	X:A ratio =1/2
	No Dosage Compensation

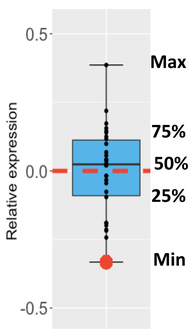


Fig. 2 Formula for the relative gene expression (*RGE*) of each autosome (black dots) and X chromosome (*RXE*; red dot). Dosage compensation definition and visualization (red line: compensated, below and above red line: incomplete and complete compensation.

Results

Table 2. NextSeq 500 sequencing summary.

Sequencing Run	Library #	Raw Reads (Million)	Total Reads After QC (Million)	Average # mapped Reads per library (Million)	Average mapping efficiency (%)
1	24	1,160	1,048	20	90
2	11	576	542	22	90
3	10	413	388	18	91
Total	**45**	**2,149**	**1,978**	**20**	**90.3**

I. Incomplete X chromosome dosage compensation in sheep major organs and embryos

Fig. 3a *RXEs* of sheep major organs and embryos

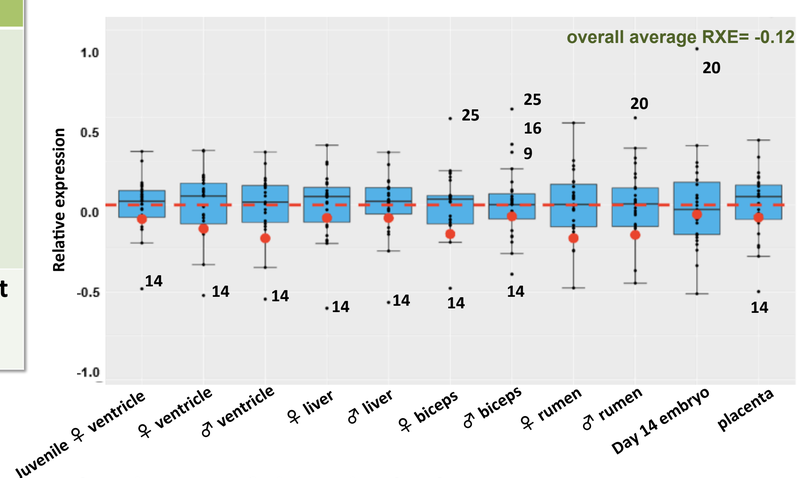


Fig. 3b *RXEs* of sheep brain tissues

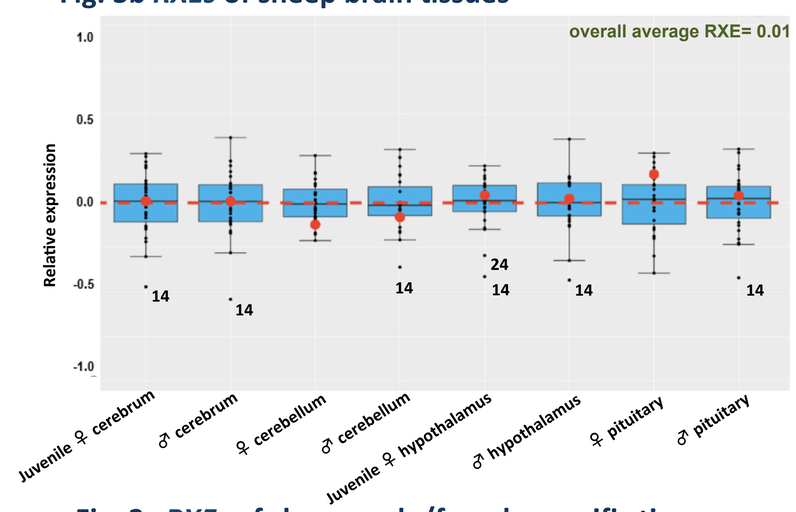
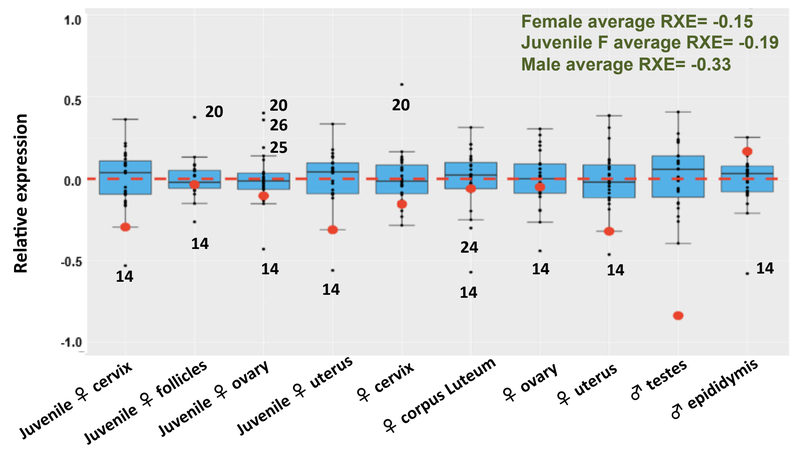


Fig. 3c *RXEs* of sheep male/female specific tissues



II. Incomplete X chromosome dosage compensation in sheep nutritional groups

Fig. 4a Control group tissues

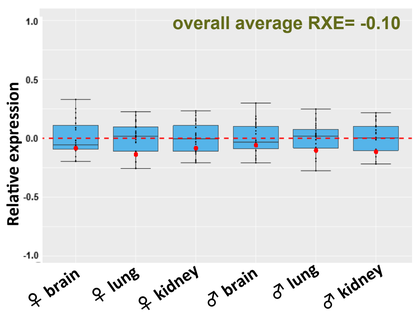


Fig. 4b Overfed group tissues

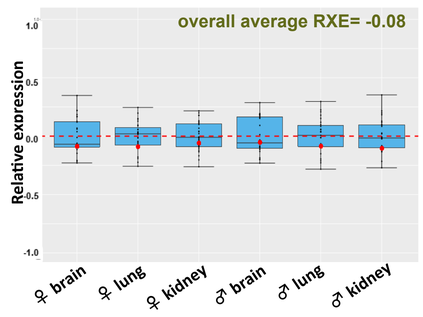
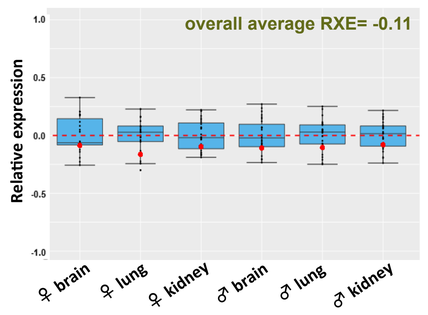


Fig. 4c Restricted group tissues



Conclusions

Our results indicate that a mechanism for dosage compensation exists in the sheep, although it is largely incomplete. Different maternal nutrition did not affect dosage compensation by Day 135 of gestation.

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