

# 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 up-regulates the active 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, XXXX:AAAA (diploid immature oocyte with DNA duplication), XX:AA (haploid mature oocyte with DNA duplication), XX:AA and X:AA (gradual changed X status in bovine pre-implantation conceptuses), and X:AA (extra-embryonic tissues and somatic cells in female 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 autosome genes were used to calculate the relative X to autosomal gene (A) expression (*RXE*):  $\log_2(X \text{ expression}) - \log_2(A \text{ expression})$  (Figure 3).

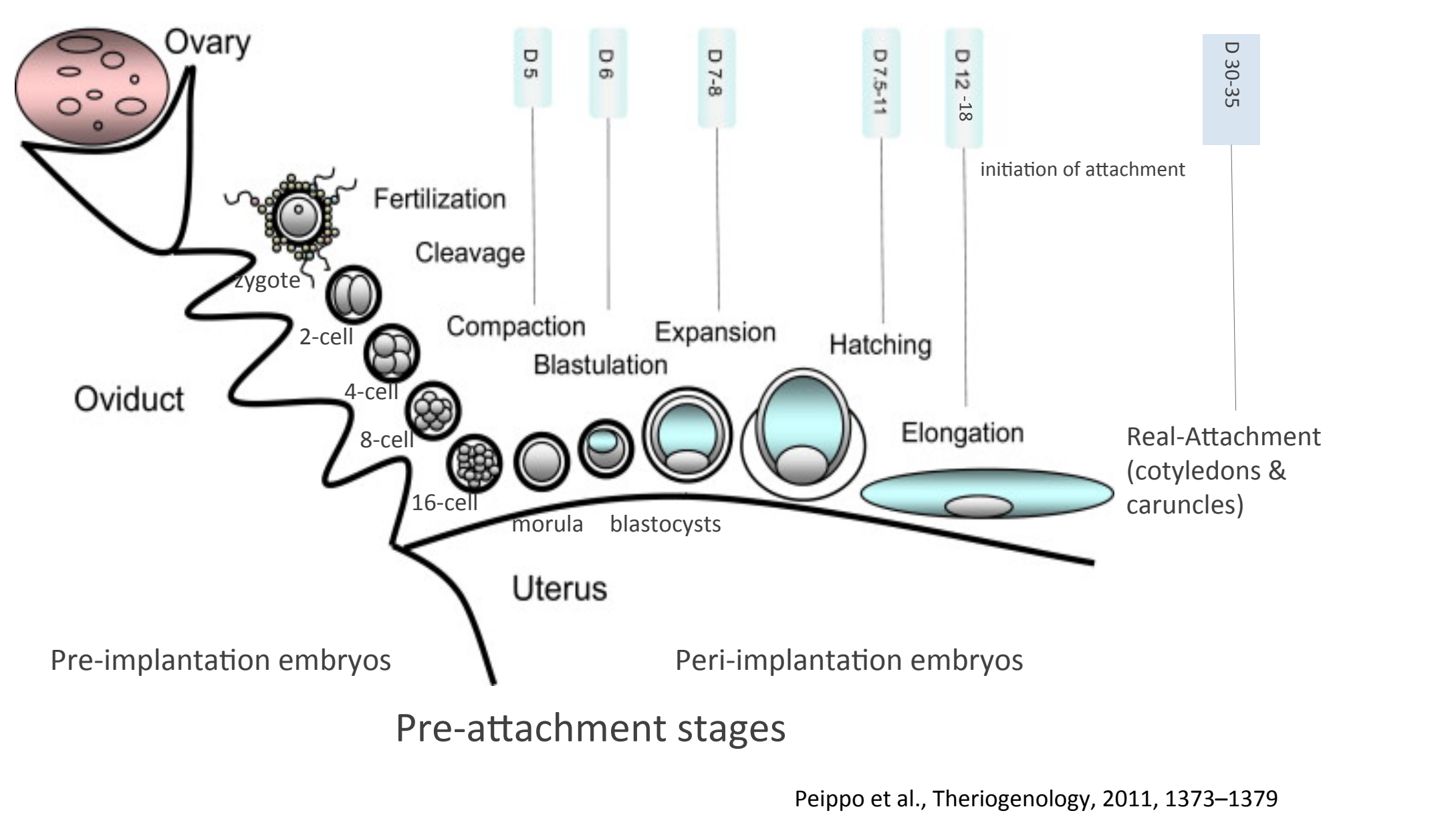


Figure 1. Bovine pre-attachment embryo development

Table 1. RNA-seq libraries used in the study.

	Tissue Type	Number of samples	Library type	BioProject ID	Reference
1	<i>In vivo</i> mature oocyte and embryo (2-, 4-, 8-, 16-, 32-cell, CM, and BL)	7*2n=14	Single-read SOLiD	PRJNA254699	Jiang et al., 2014
2	<i>In vitro</i> immature & mature oocyte and embryo (4-, 8-, 16-cell, and BL)	6*3pools=18	Single-read Illumina	PRJNA228235	Graf et al., 2014
3	Conceptuses (Day 7, 10, 13, 16, 19)	6n+7n+3*5n=28	Single-read Illumina	PRJNA243569	Loneragan Pat. et al 2011
4	Female specific tissue: Endometrium & CL	12n+14n=26	Paired-end Illumina	PRJNA298914	Moore et al., 2016
5	Male and female somatic tissues	4*4n=16 female	Single-read Illumina	PRJNA268096	Chen et al., 2015
6		7 pools male	Paired-end Illumina	PRJEB6377	PRJEB6377,2014
7		4*5n*2s=40 Male & female	Paired-end Illumina	PRJNA273164	Jeong J et al., 2016
8	Placenta (control and clone)	2 pools	Single-read Illumina	PRJNA229443	Su et al., 2014

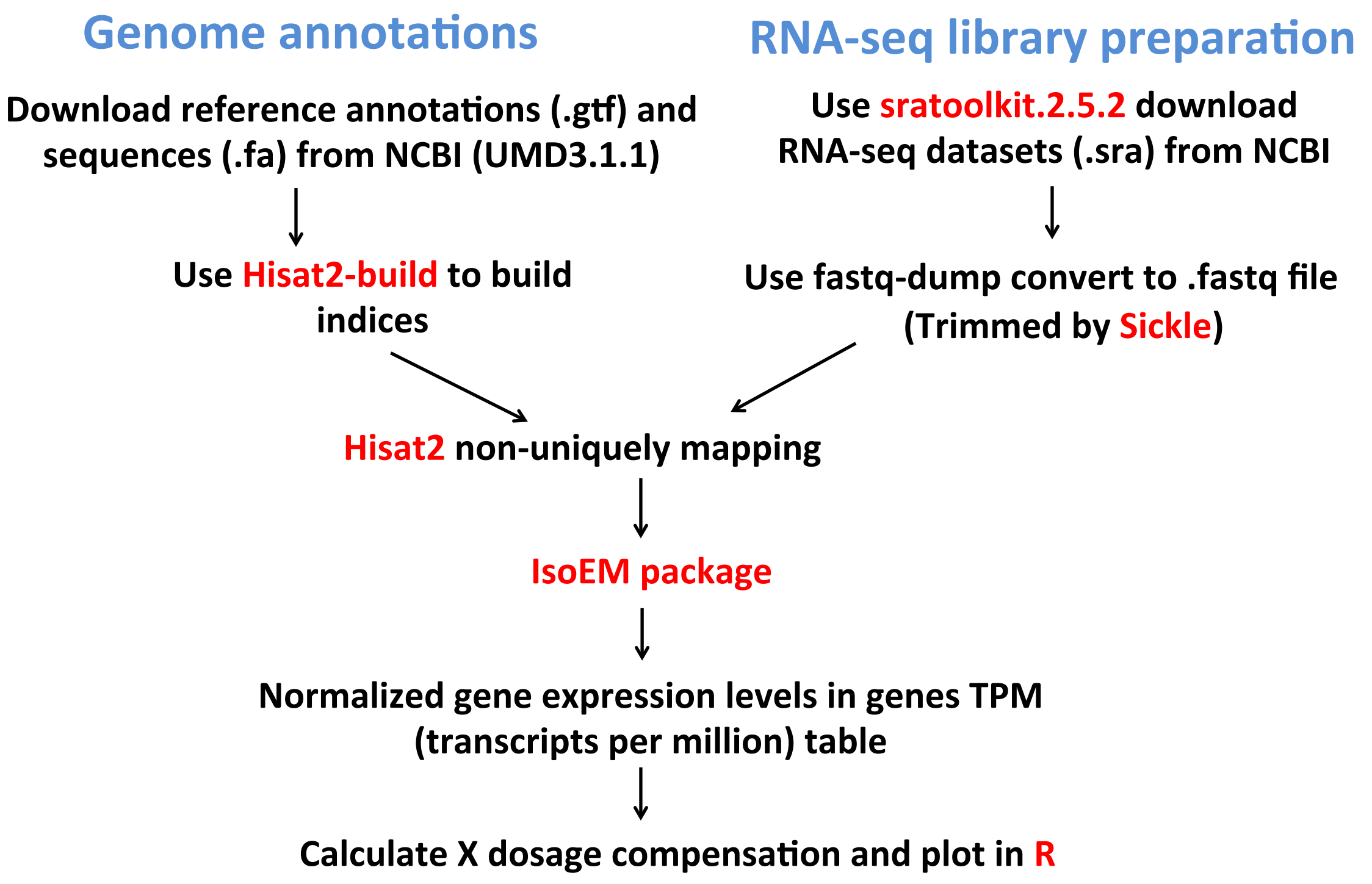


Figure 2. RNA-seq data analysis workflow. Reads were trimmed and non-uniquely (paralogs included) mapped to the bovine reference genome assembly UMD3.1.1 using Hisat2 (version 2.0.5) aligner. The mRNA level for each gene, estimated by transformed transcripts per kilobase million (TPM) was quantified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

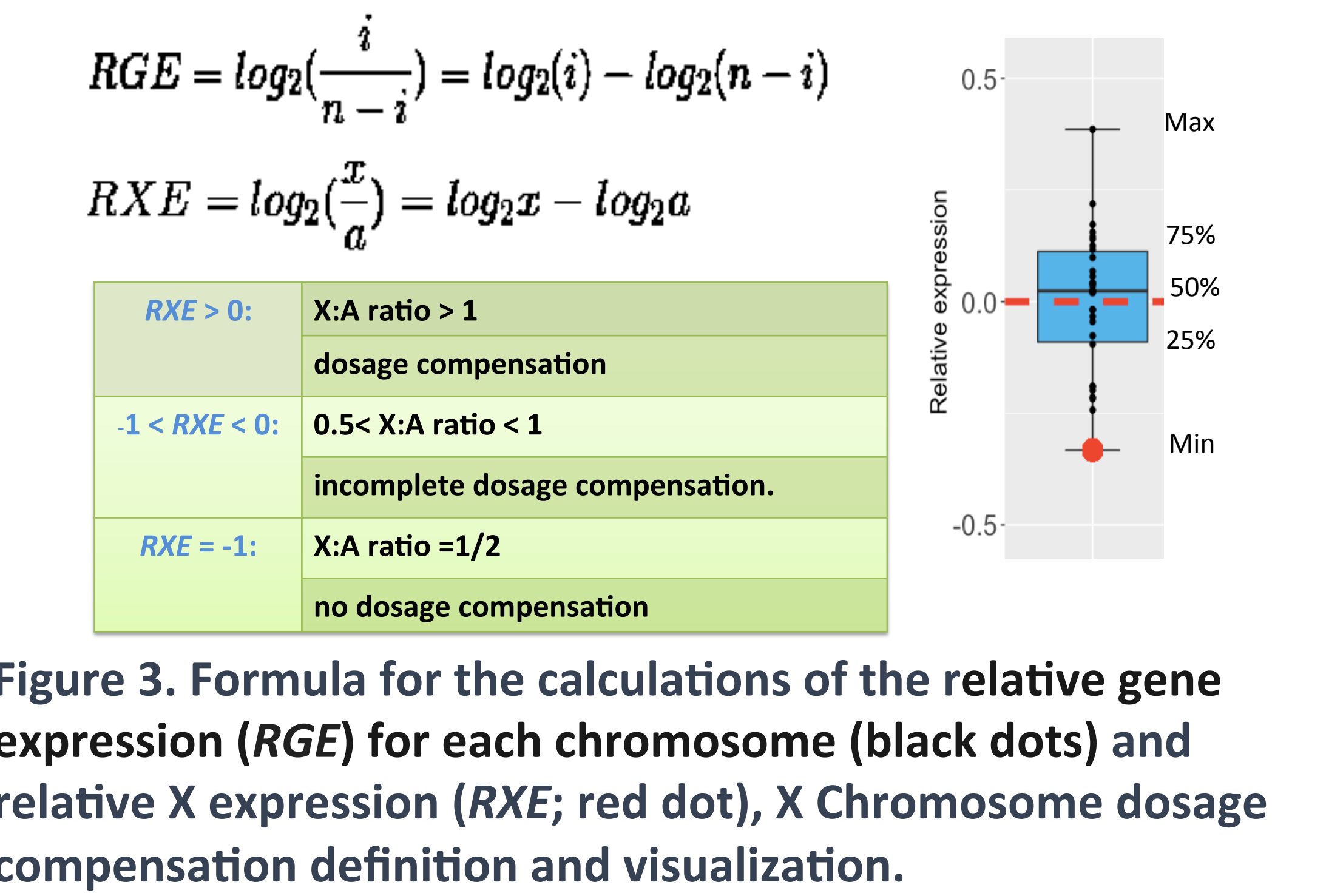


Figure 3. Formula for the calculations of the relative gene expression (*RGE*) for each chromosome (black dots) and relative X expression (*RXE*; red dot), X Chromosome dosage compensation definition and visualization.

## Results

### I. Datasets distribution overview

Table 2. The numbers and percentages of paralogs ( $\geq 70\%$  similarity) as well as the total numbers of annotated genes in each bovine chromosome.

Chromosome	Total numbers of Genes	Numbers (%) of Paralogs	Chromosome	Total numbers of Genes	Numbers (%) of Paralogs
1	985	167 (0.17)	16	710	129 (0.18)
2	1021	229 (0.22)	17	665	149 (0.22)
3	1372	314 (0.23)	18	1236	207 (0.17)
4	855	222 (0.26)	19	1347	303 (0.22)
5	1323	336 (0.25)	20	384	91 (0.24)
6	692	156 (0.23)	21	731	221 (0.30)
7	1396	377 (0.27)	22	608	110 (0.18)
8	829	230 (0.28)	23	785	264 (0.34)
9	602	146 (0.24)	24	347	98 (0.28)
10	1074	316 (0.29)	25	766	102 (0.13)
11	1047	192 (0.18)	26	437	82 (0.19)
12	414	98 (0.24)	27	274	73 (0.27)
13	850	185 (0.22)	28	355	78 (0.22)
14	571	135 (0.24)	29	705	186 (0.26)
15	1050	387 (0.37)	X	1128	374 (0.33)
Genome Average	819	199 (0.24)			

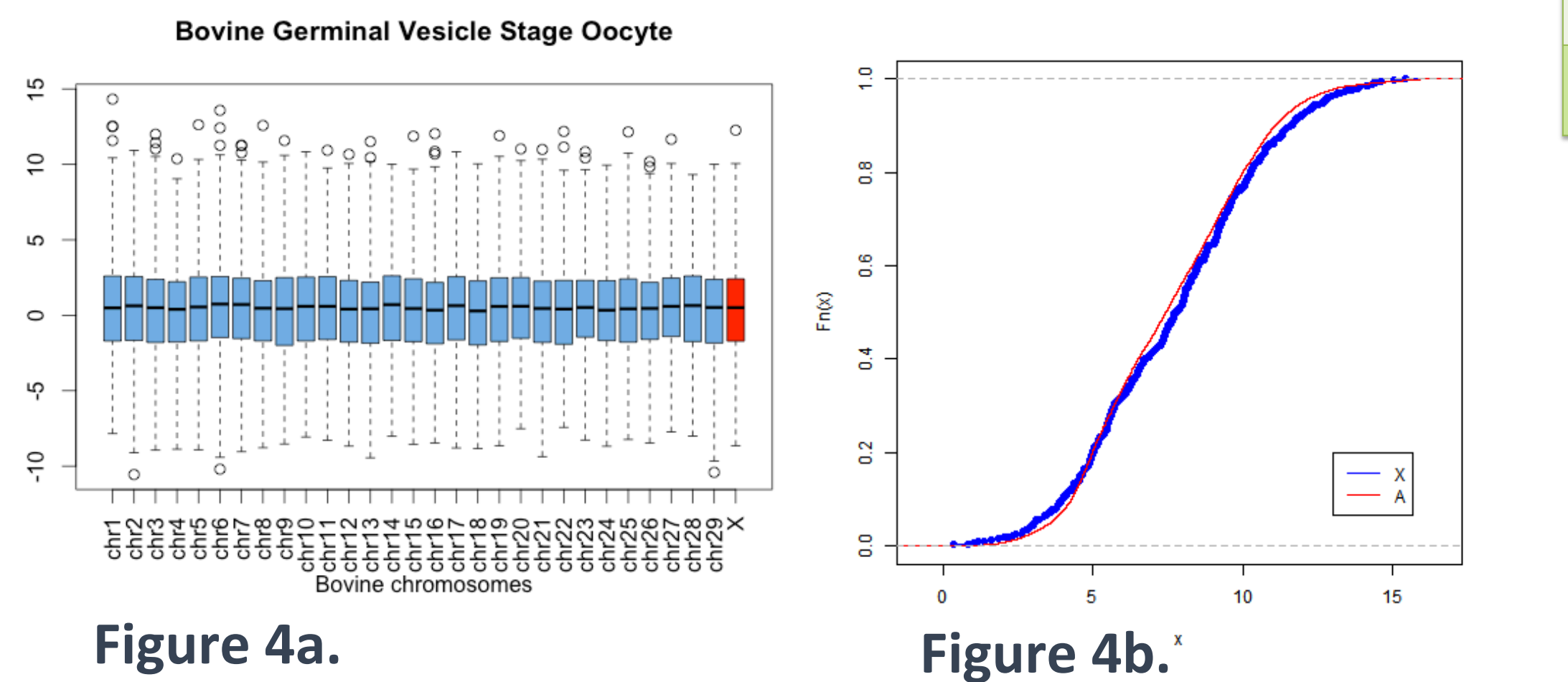


Figure 4. Expression ranges of bovine chromosomes in representative bovine immature oocytes. a. The ranges and medians of X-linked gene expression (red; TPM > 0) were similar to those of autosome pairs (blue). b. A representative empirical cumulative distribution (ECD) plot showing that the distribution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

## II. Incomplete X chromosome dosage compensation in bovine germline, early embryos, and somatic tissues

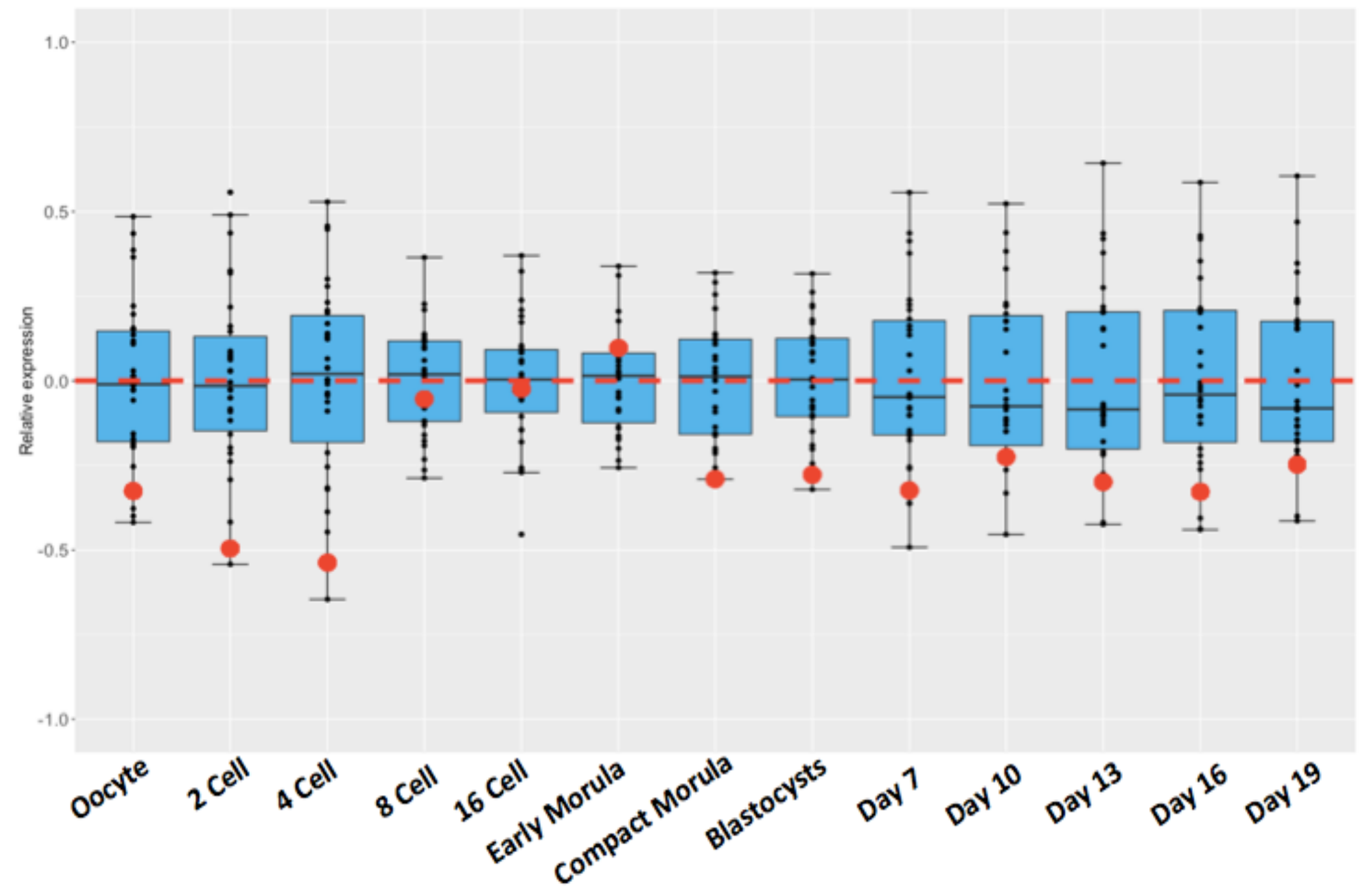


Figure 5a. *RXEs* (red dots) in in vivo matured oocytes and embryos.

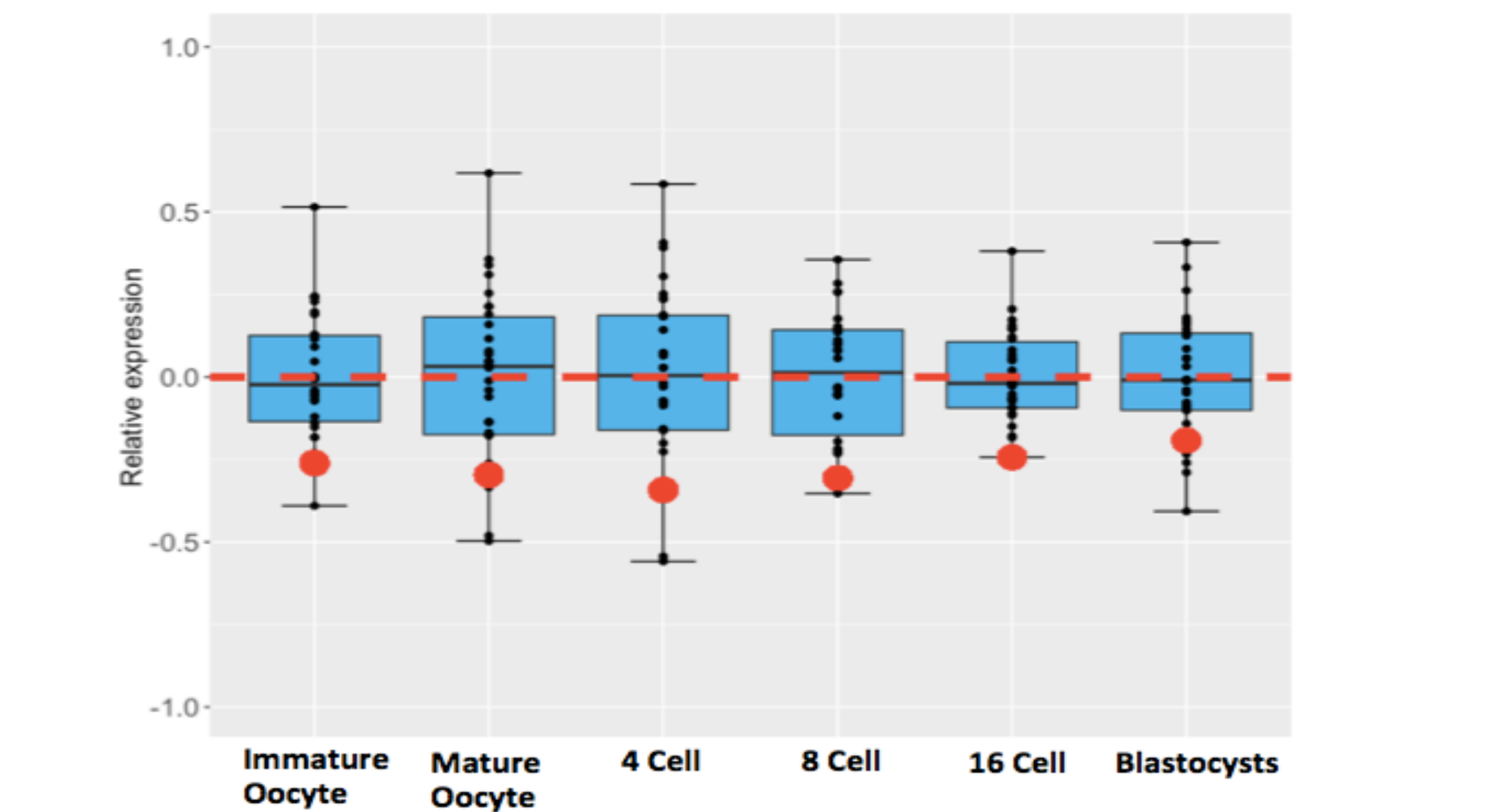


Figure 5b. *RXEs* in in vitro immature/mature oocyte and embryos.

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*.

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

<i>RXE</i> value	Brain	Muscle	Liver	Kidney	Fat	Duodenum	Lung	Endometrium	Corpus Luteum	Placentome
Female	-0.07	-0.25	0.01	-0.18	-0.22	NA	NA	-0.33	-0.17	Natural: -0.13 Cloned: -0.20
Male	-0.09	-0.25	-0.10	-0.12	-0.25	-0.21	-0.40	NA	NA	NA
Average	-0.08	-0.25	-0.05	-0.15	-0.23	-0.21	-0.40	-0.33	-0.17	-0.17

## 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

USDA-ARS: 1265-31000-091-02S; NIFA-USDA: W2171/3171; National Natural Science Foundation of China: 30760167; Department of Education of Xinjiang Uygur Autonomous Region Scholarship: 2016-3-0036.