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Christian-Albrechts-Universität zu Kiel
Institut für Tierzucht und Tierhaltung

Characterization of sex chromosomes-linked lncRNAs in Holstein bull spermatozoa under stress conditions

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Season 79, Improved insight into the reproduction physiology of livestock

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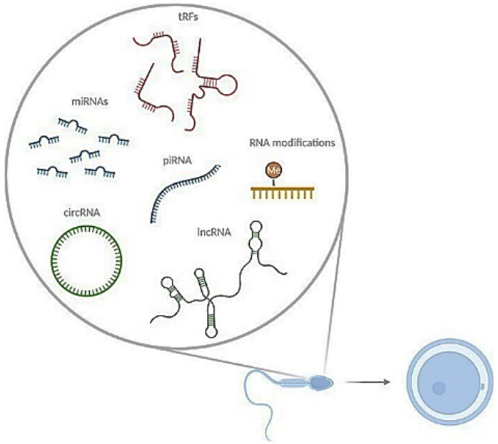
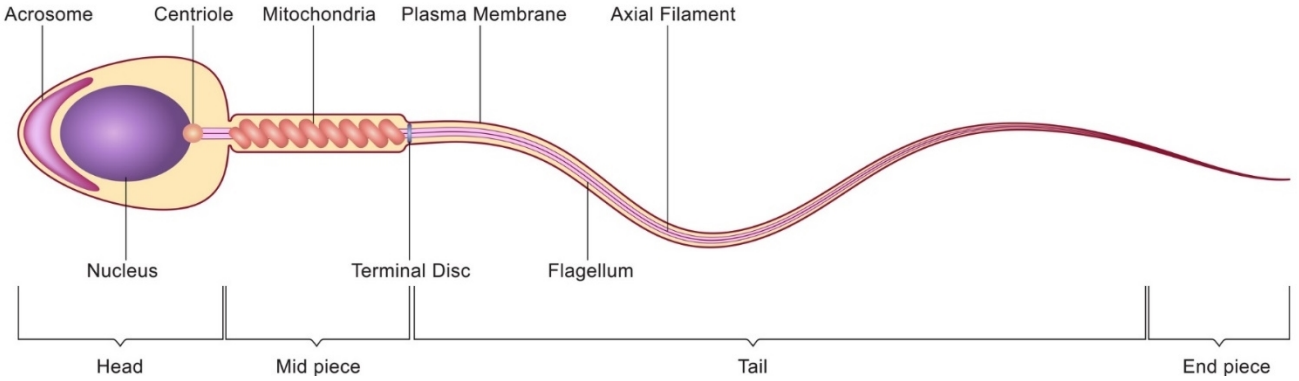
Sperm structure

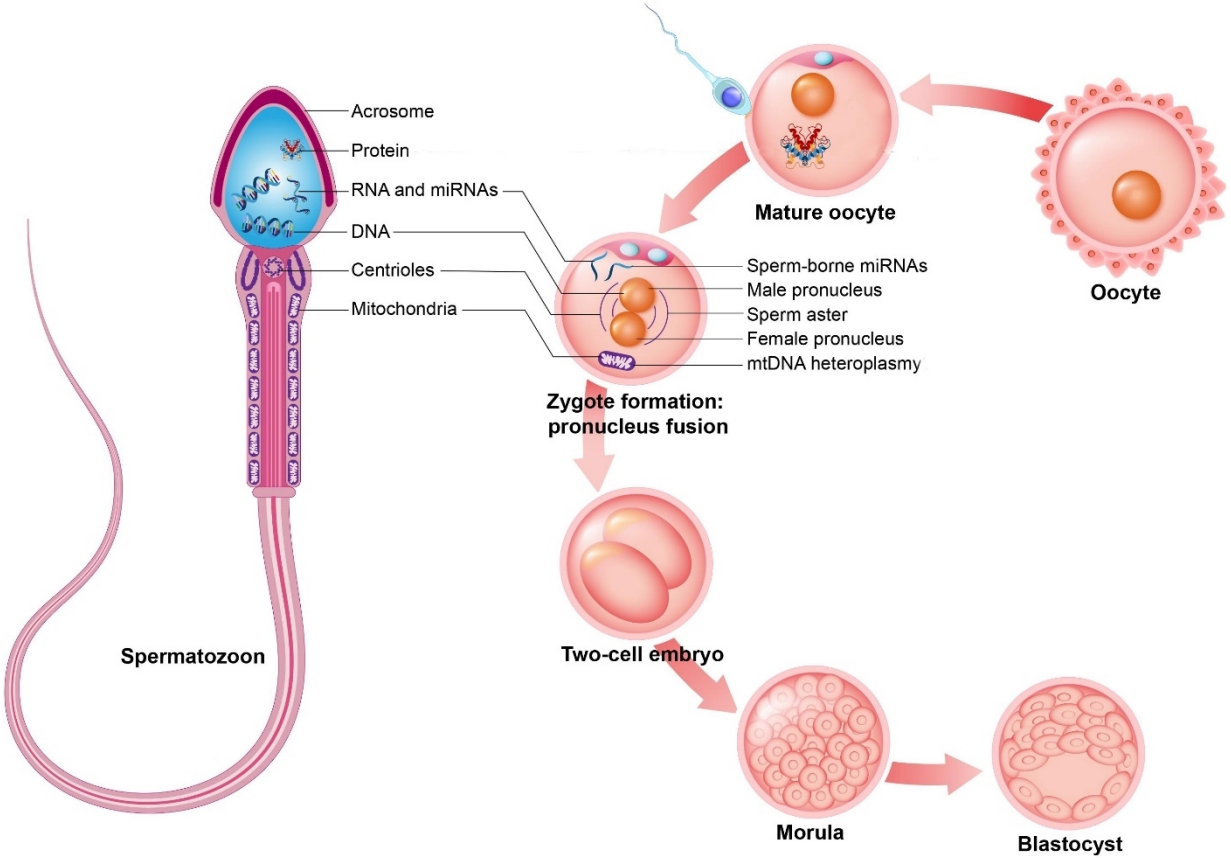




Sperm head contains DNA (nuclear 30 chromosomes), and different molecular molecules.

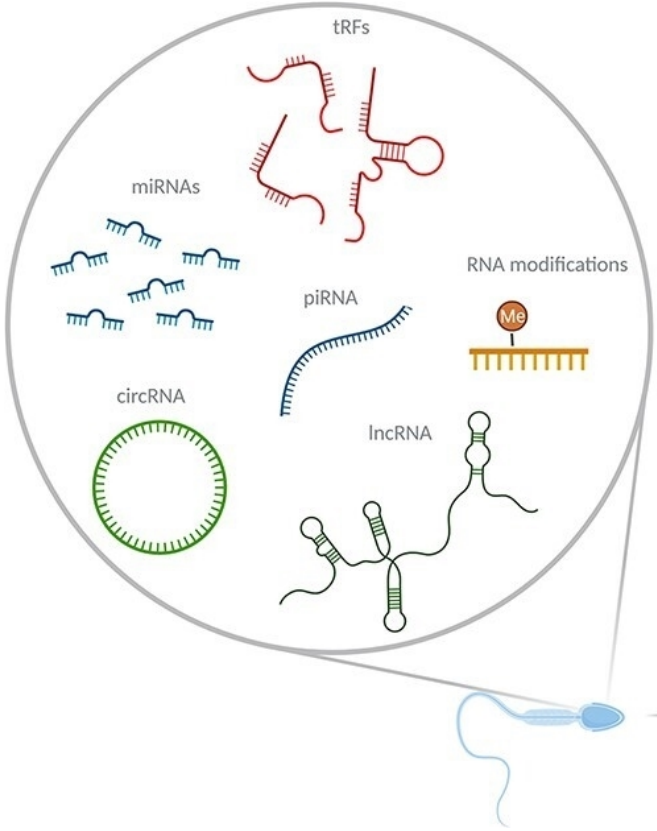
Kretschmer and Gapp, 2022

Sperm contribution in fertilization and early embryonic development success

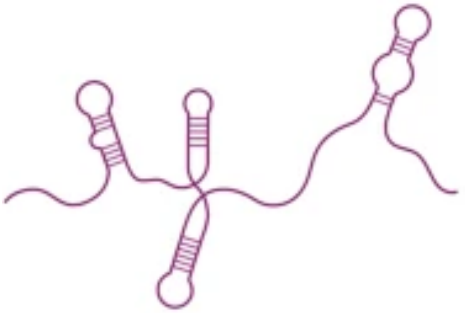


Alves et al., 2020

Sperm-borne RNAs



- Long non-coding RNAs (lncRNAs) have a size of more than 200 non-protein codon nucleotides, expressed in most types of cells, and are known to regulate cellular functions on the level of DNA, RNA, and protein.

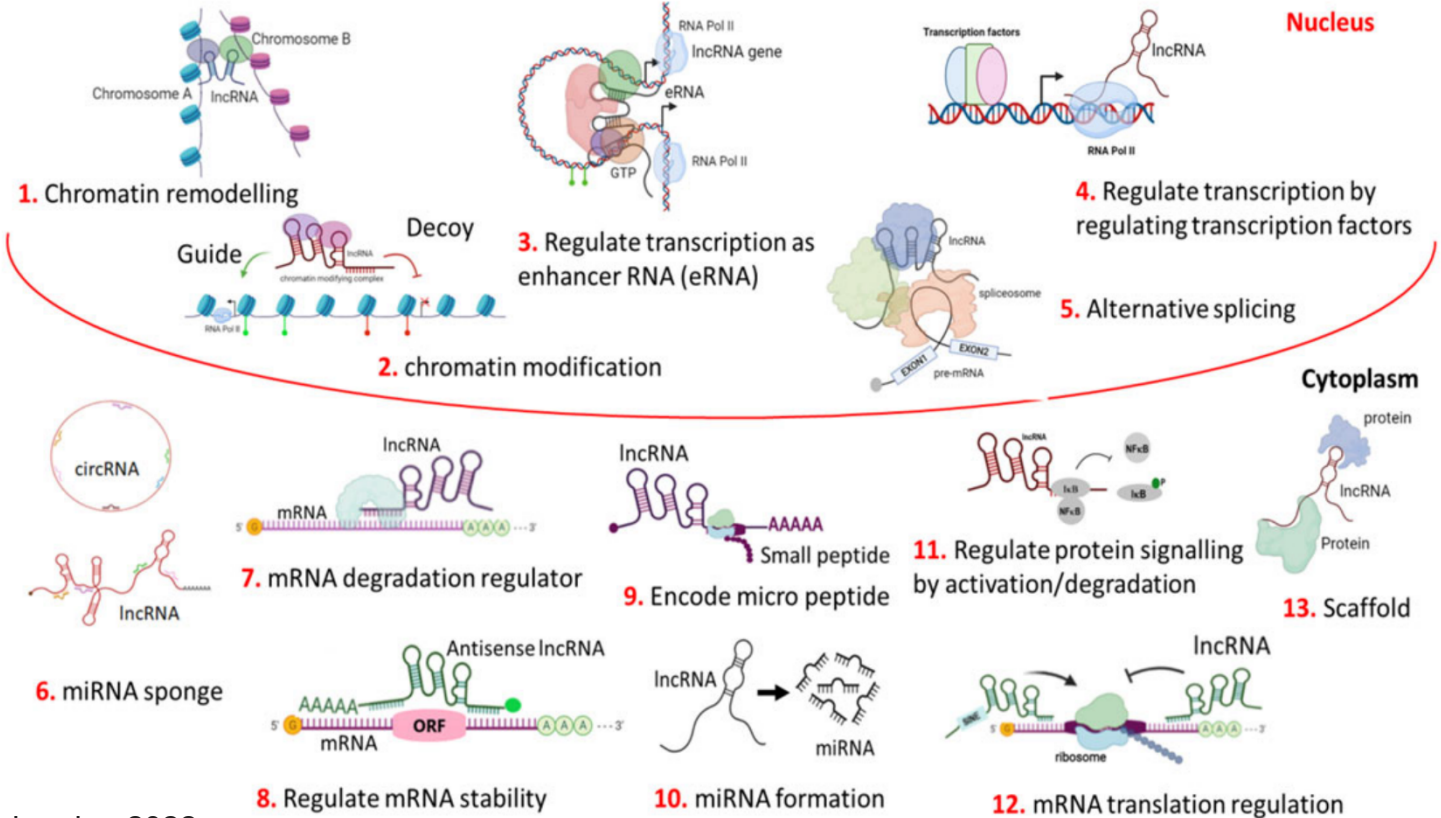


Long ncRNAs
(> 200 nucleotides)

Mature oocyte

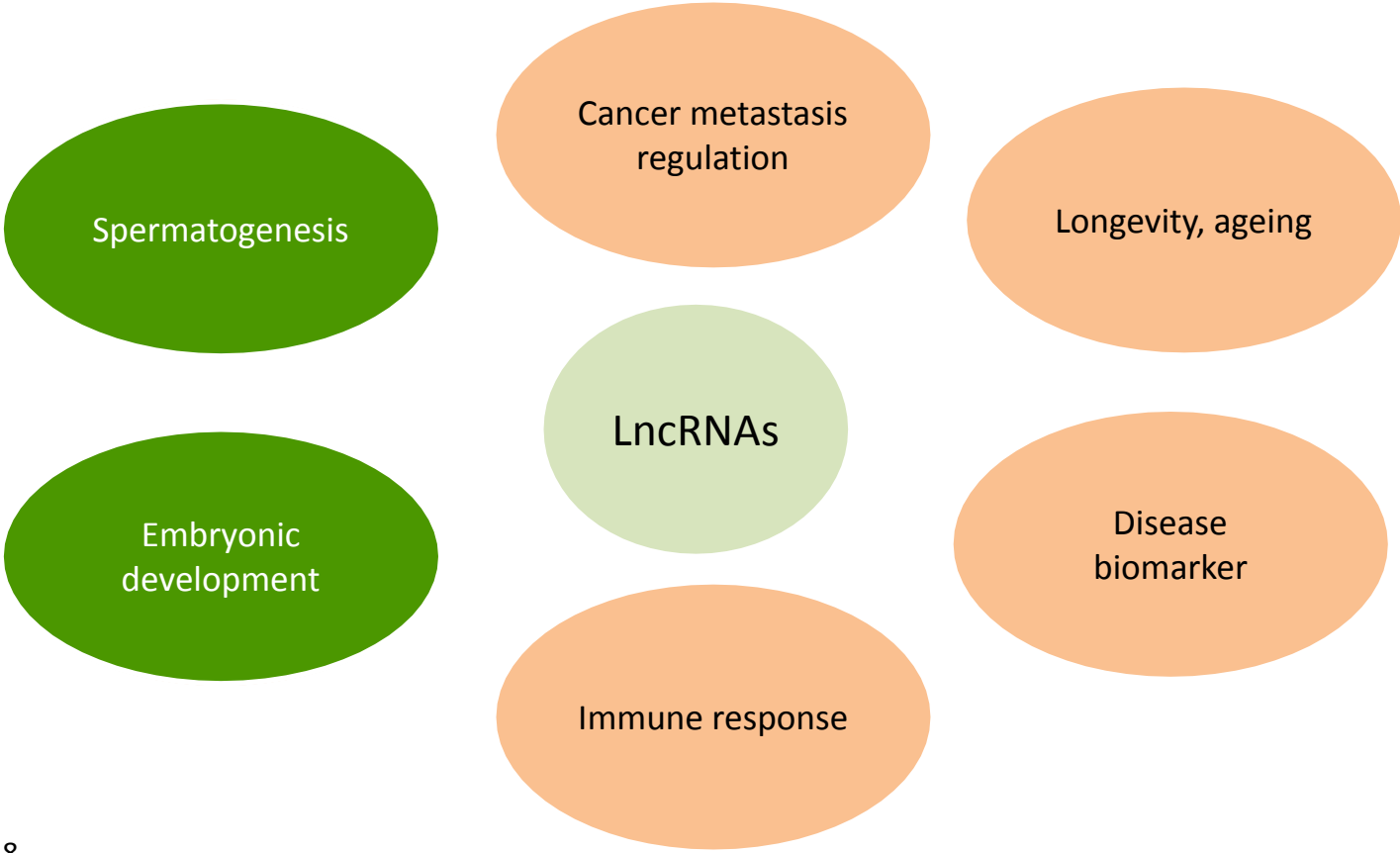
Kretschmer and Gapp, 2022

Long non-coding RNAs functions



Ghosh and Majumder, 2022

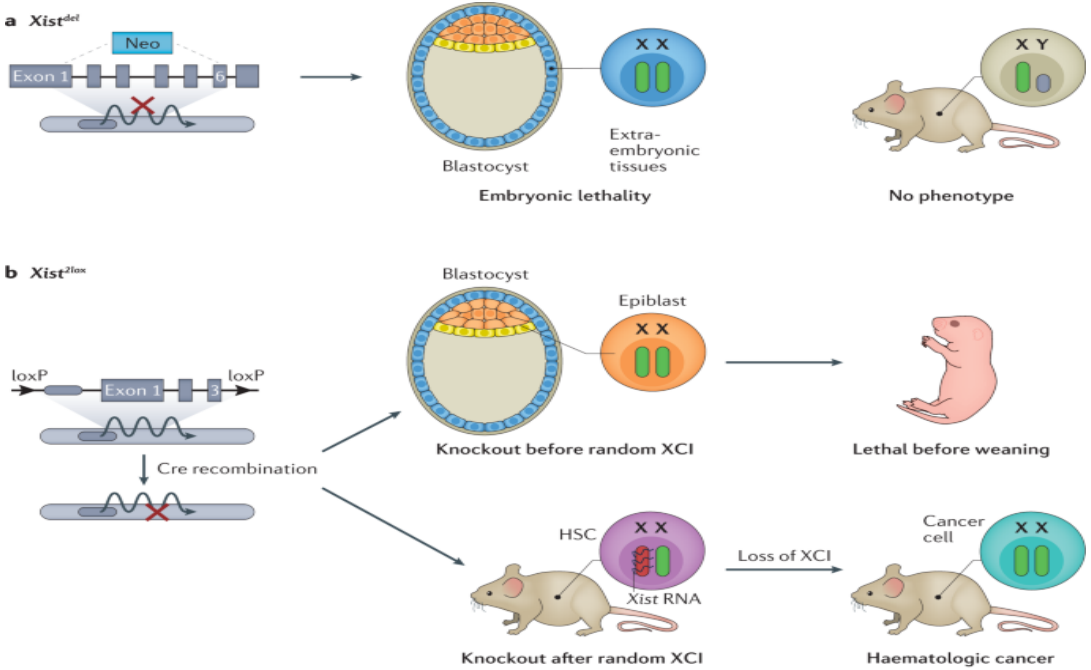
Potential biological roles of lncRNAs



Dhanoa et al., 2018

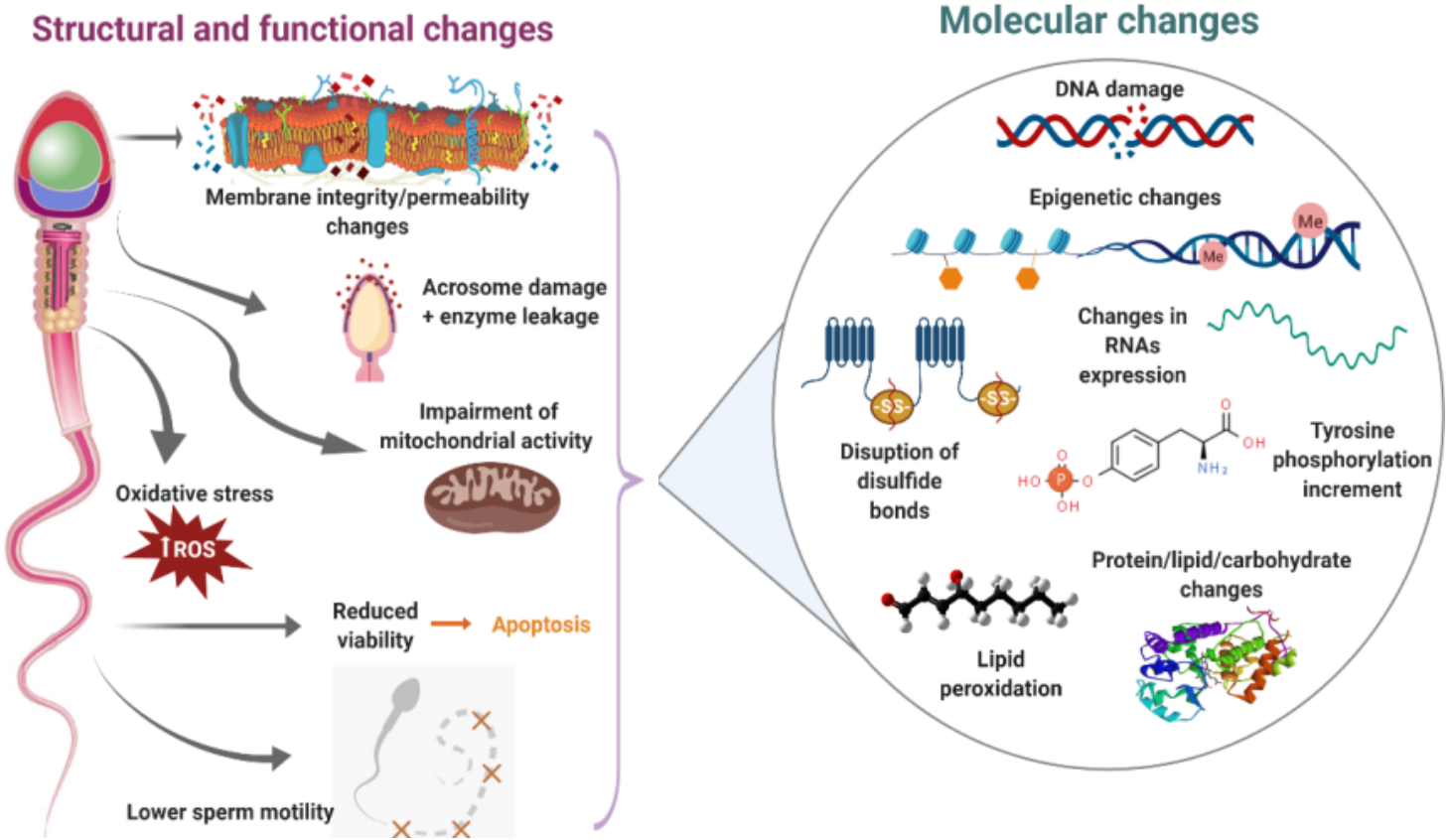
Deletion of *XIST* lncRNA led to newborn female-lethal

- X- inactivation specific transcript (*XIST*) gene is located in the X inactivation center (XIC)
- *XIST* is expressed in females and males
- *XIST* plays important roles in the differentiation, proliferation, and genome maintenance



Andergassen and John, 2022; Jonkers et al., 2008

Stress negatively influences sperm structural and molecular features



Peris-Frau et al., 2020

Research gap and objectives

Characterization of bovine lncRNAs and their potential roles in sperm functions remain elusive

The main goals of the current study are to:

- Investigate the expression pattern of sperm-borne-lncRNAs located at sex chromosomes
- Identify the potential role of sperm-borne-lncRNAs on sperm quality and subsequent embryo development

Expermintal design I

Semen (8 bulls, Holstein)

Qualified (4-5 years)

Non-quallified (1 year)

Winter, Spring, Summer, and Autumn

Sperm cells

DNA isolation

PCR amplification

Genotyping of *TMSB15B*-upstream region using Sanger seq

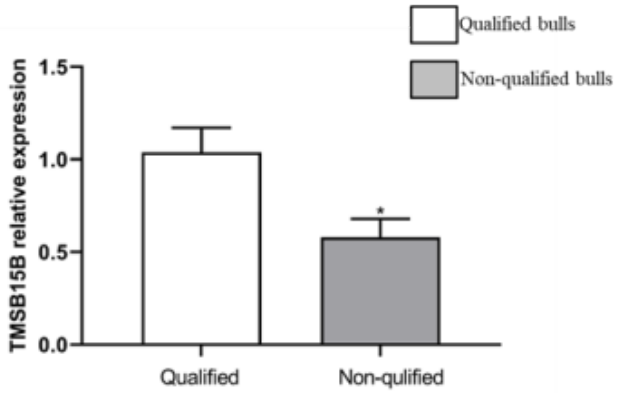
RNA isolation

cDNA synthesis

Quantification analysis of sex-chromosomes lncRNAs (*XIST, JPX, TMSB15B, and KANTR*)

Higher RNA level of *TMSB15B* lncRNA in qualified bulls

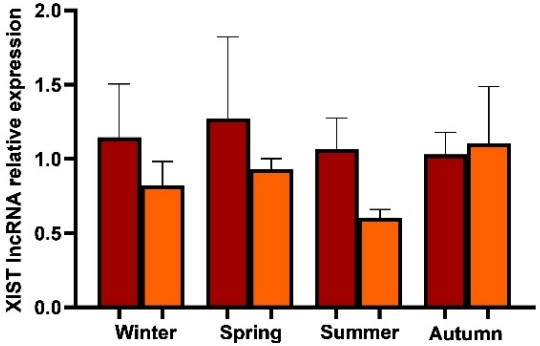
Qualified bulls	SNP	Non-qualified bulls	SNP
Reference	G	**Reference**	G
Bull #1	A	**Bull #1**	G
Bull #2	A	**Bull #2**	G
Bull #3	G	**Bull #3**	G
Bull #4	G	**Bull #4**	G
Bull #5	G	**Bull #5**	G
Bull #6	G	**Bull #6**	G



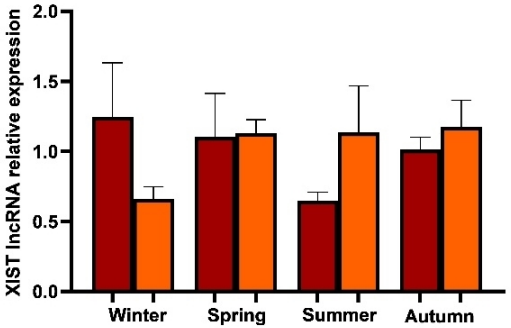
Data are mean ± SE, * p < 0.05, Thymosin beta 15B (*TMSB15B*)

Differential RNA level of XIST lncRNA in qualified and non-qualified bulls

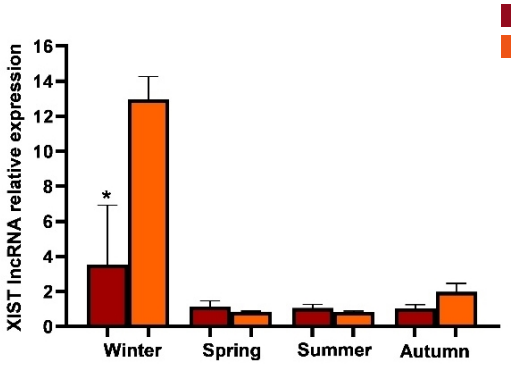
Overall *XIST* lncRNA in the same season



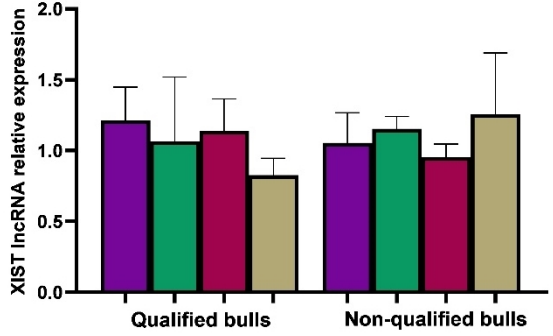
XIST lncRNA transcript 2 in the same season



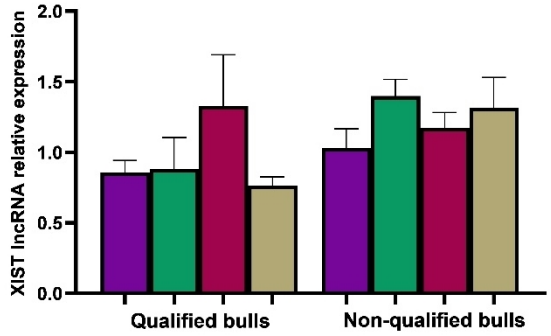
XIST lncRNA transcript 2 and 3 in the same season



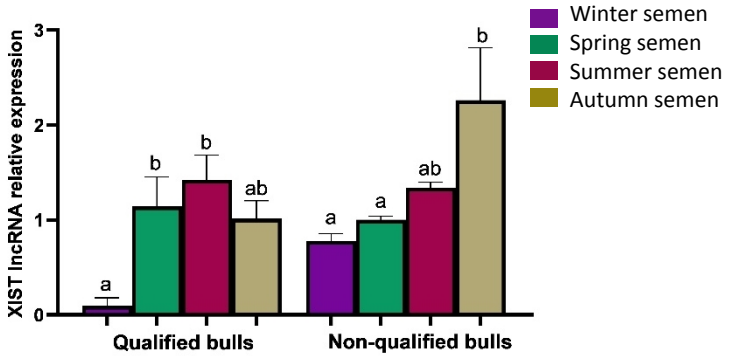
Overall *XIST* lncRNA in different seasons



XIST lncRNA transcript 2 in different seasons



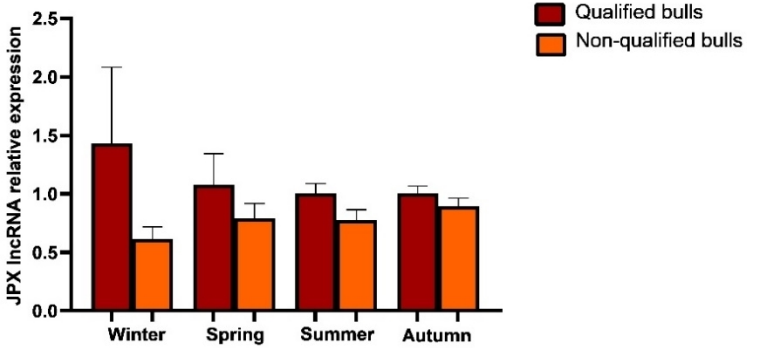
XIST lncRNA transcript 2 and 3 in different seasons



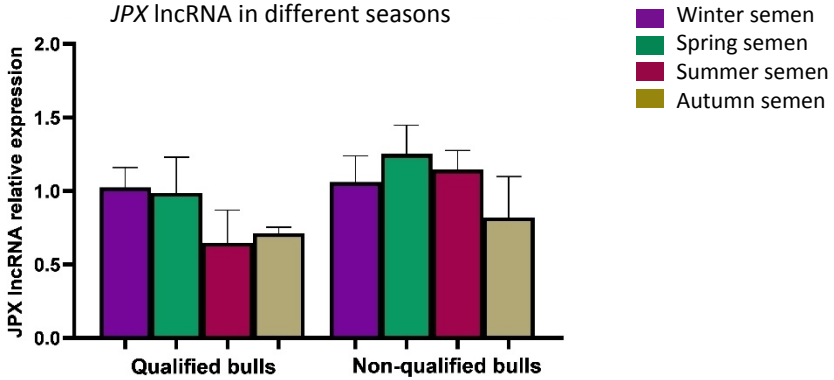
Data are mean ± SE, *, a, b $p < 0.05$

Season had no effect of the RNA level of *JPX* and *KANTR* lncRNAs

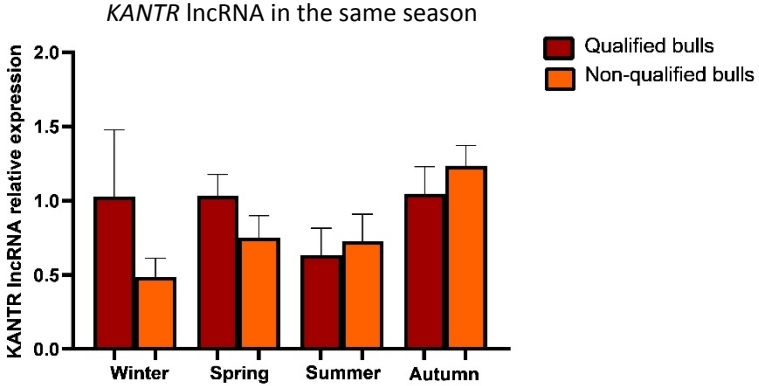
JPX lncRNA in the same season



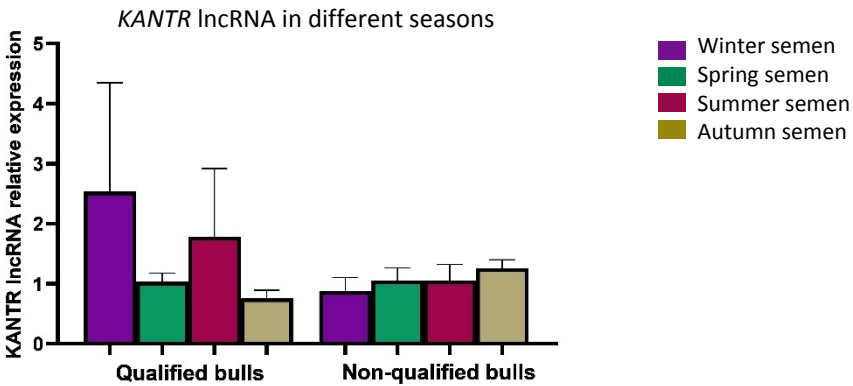
JPX lncRNA in different seasons



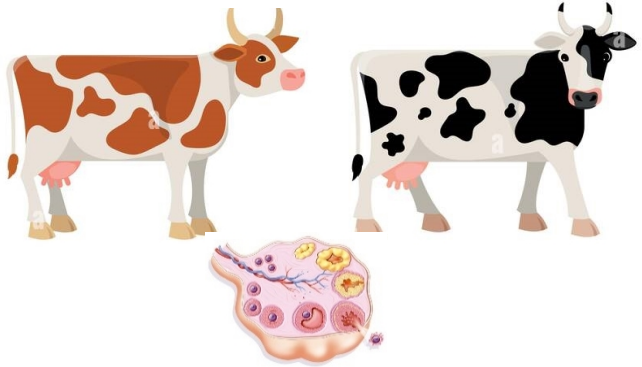
KANTR lncRNA in the same season



KANTR lncRNA in different seasons



Data are mean ± SE



Oocytes

↓

RNA isolation

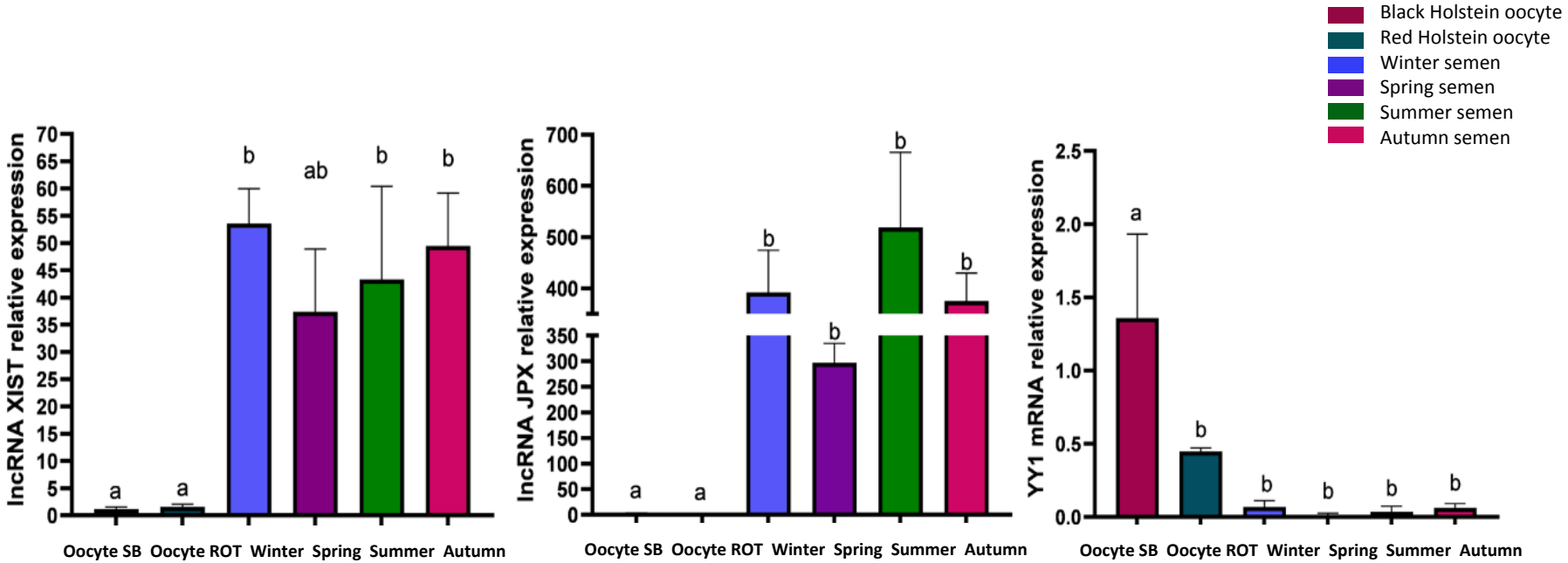
↓

cDNA synthesis

↓

Quantification analysis of sex-chromosomes lncRNAs *(XIST, JPX, and YY1*)

Lower expression level of *XIST* and *JPX* lncRNAs in oocytes compared to sperm cells



Data are mean ± SE, a, b $p < 0.05$

Take home message

- The private variant A/G in *TMSB15B* correlates with the diffrential expression level between qualified and non-qualified bulls
- Seasons have no effect on candidate sperm-borne-lncRNAs content
- Sperm-borne-lncRNAs may have the ability to be transferred to the oocyte during fertilization and are subsequently involved in regulating gene expression and cellular processes in the early embryo development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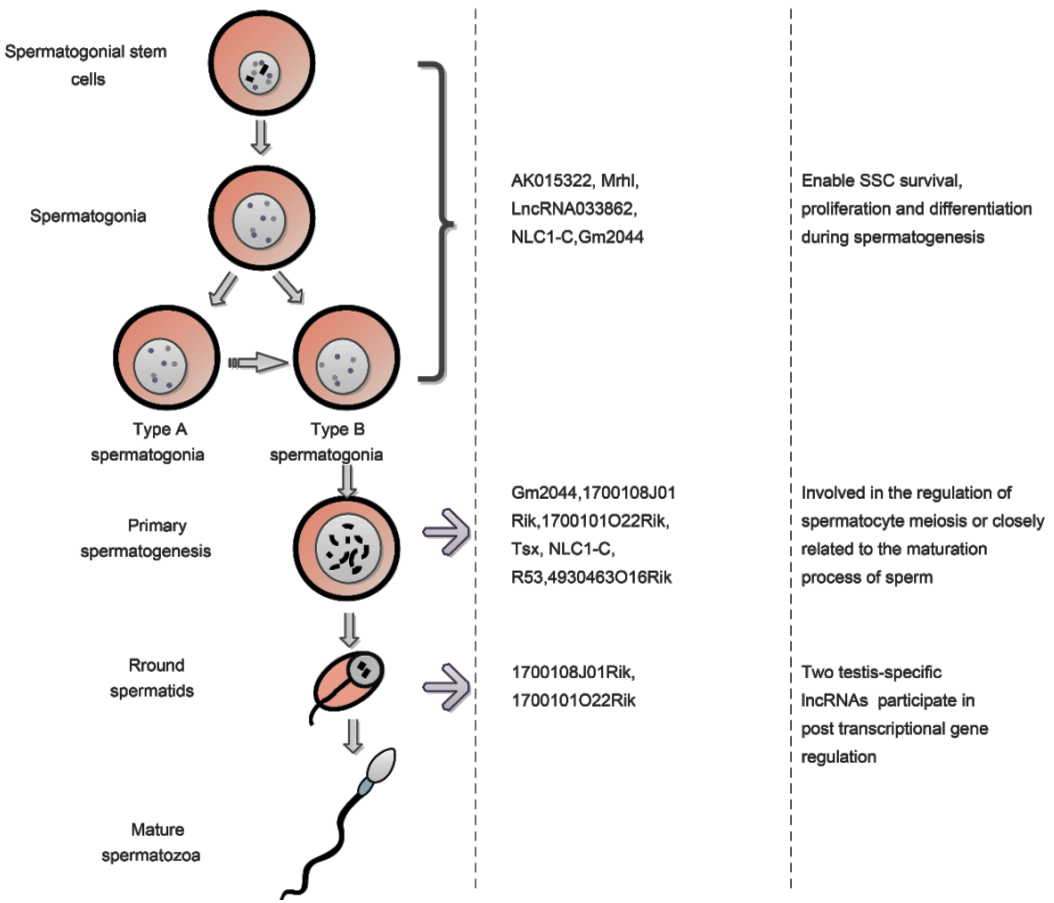
Thank you for your attention

LncRNAs roles in the regulation of spermatogenesis



Zhao et al., 2021