

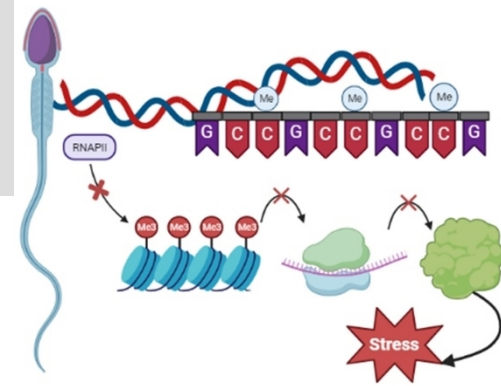


## Non-coding exonic microsatellite in bovine *Nrf2* gene influences sperm oxidative stress capacity

Khurshaid Anwar, Georg Thaller, Mohammed Saeed-Zidane

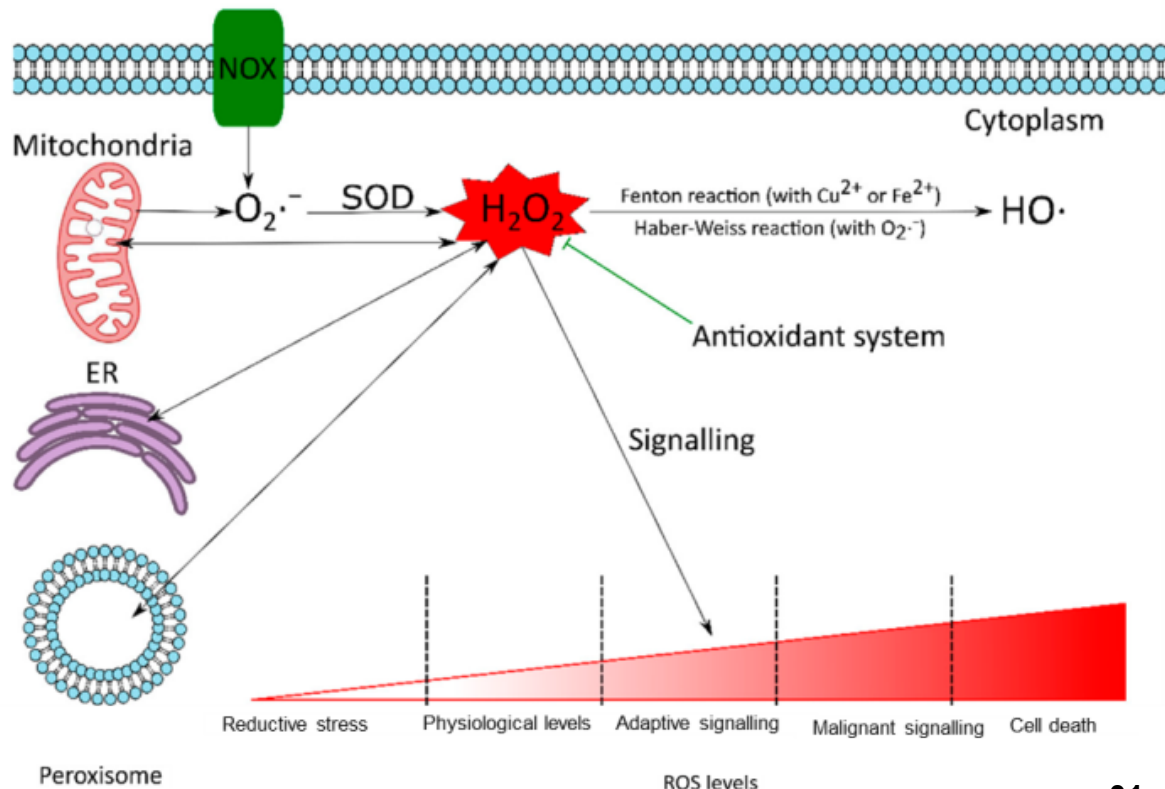
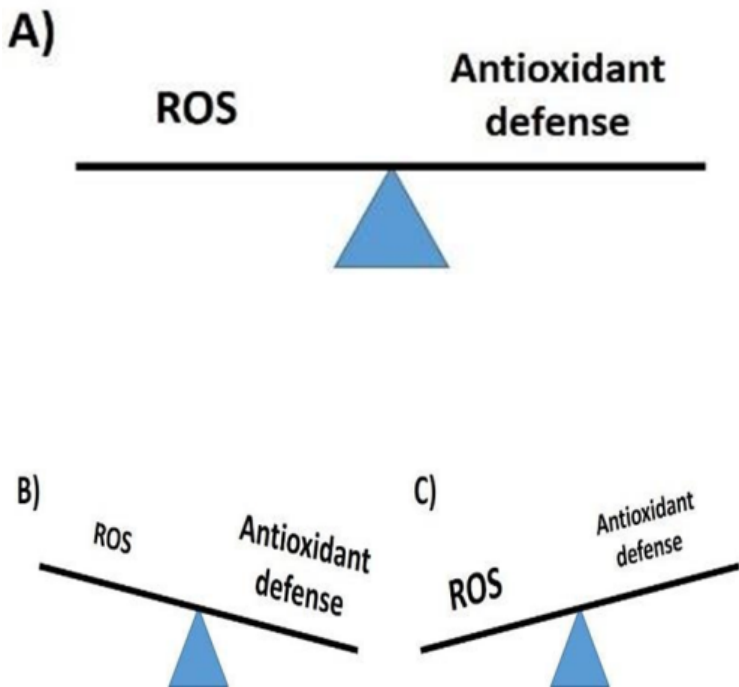
Institute of Animal Breeding and Husbandry  
Christian-Albrechts-University, Kiel, Germany  
EAAP – Programme of the 74th annual meeting  
August 26th / September 1st, 2023 Lyon, France.

Session 10, Impact of epigenetics and genetics in determining animal physiology, Abstract number 42976  
kanwar@tierzucht.uni -kiel.de

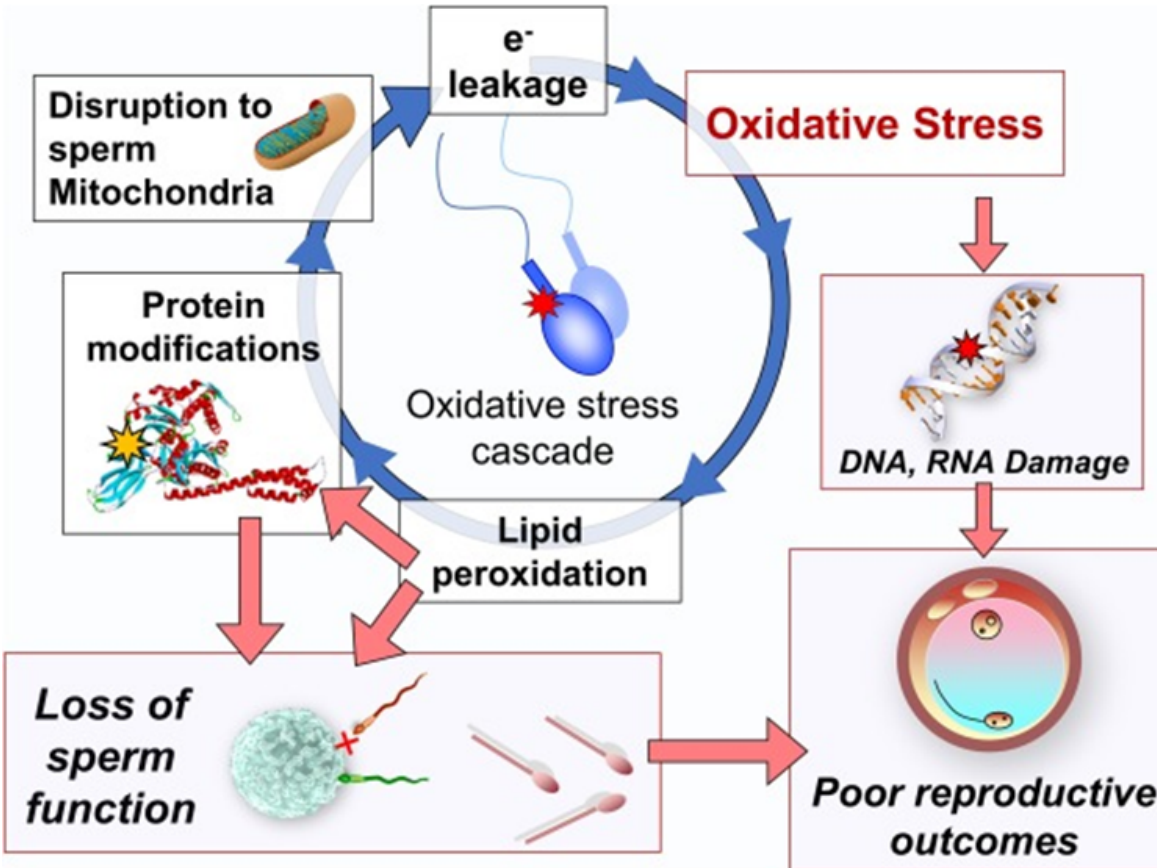


# Oxidative stress

Oxidative stress refers to the imbalance between the reactive oxygen species (ROS) and antioxidant elements.



# Oxidative stress on bull sperm



## Endogenous Sources

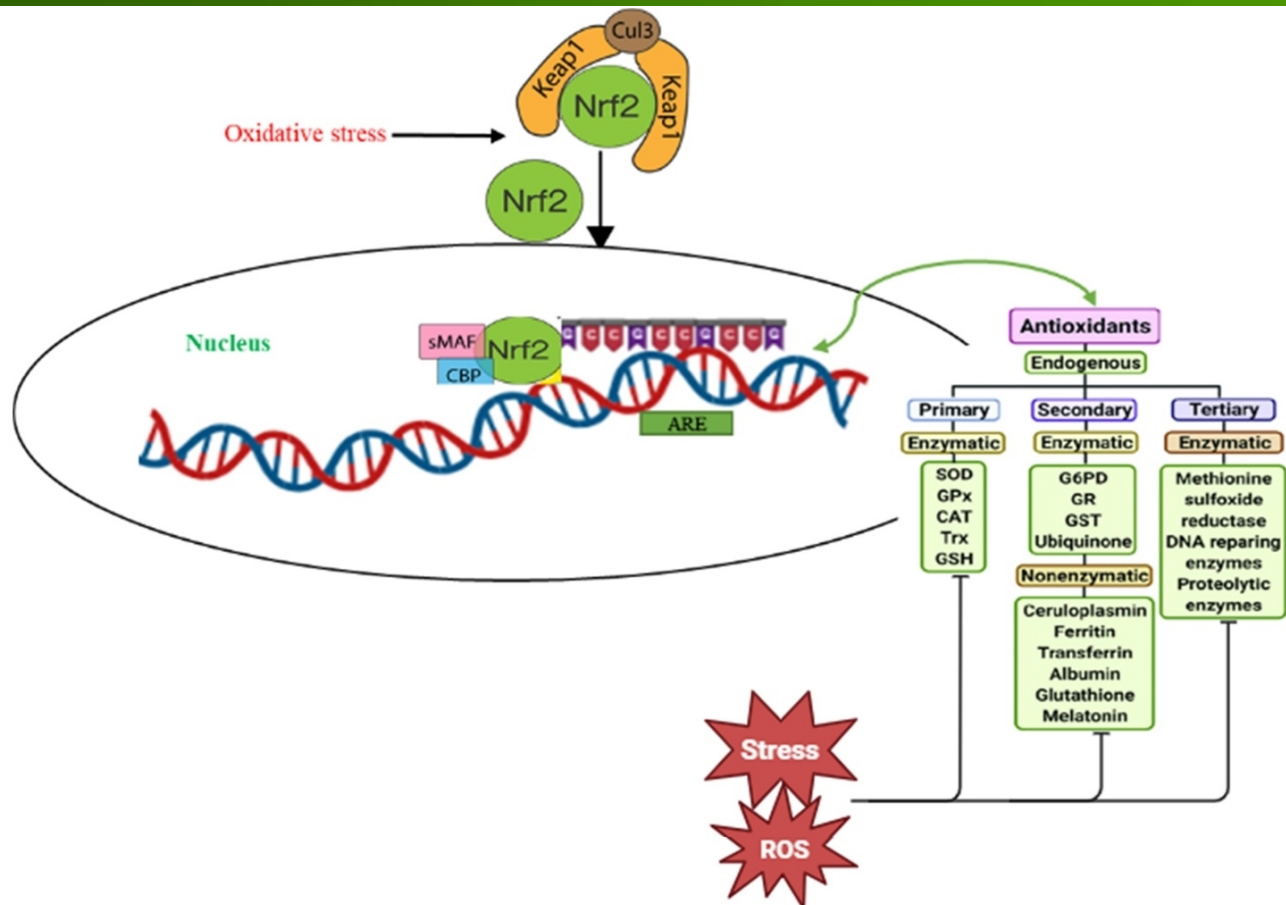
- Inflammation
- Oxidative phosphorylation
- Mitochondrial dysfunction
- NADPH Oxidase
- Xanthine oxidase
- Myeloperoxidase
- Nitric oxide synthase (NOS)
- Cytochrome P450 oxidases
- Endoplasmic reticulum

## Exogenous Sources

- Environmental Pollutants
- Radiation
- UV Radiation
- Heat Exposure
- Medications and Drugs
- Pathogens



# Cellular response to oxidative stress

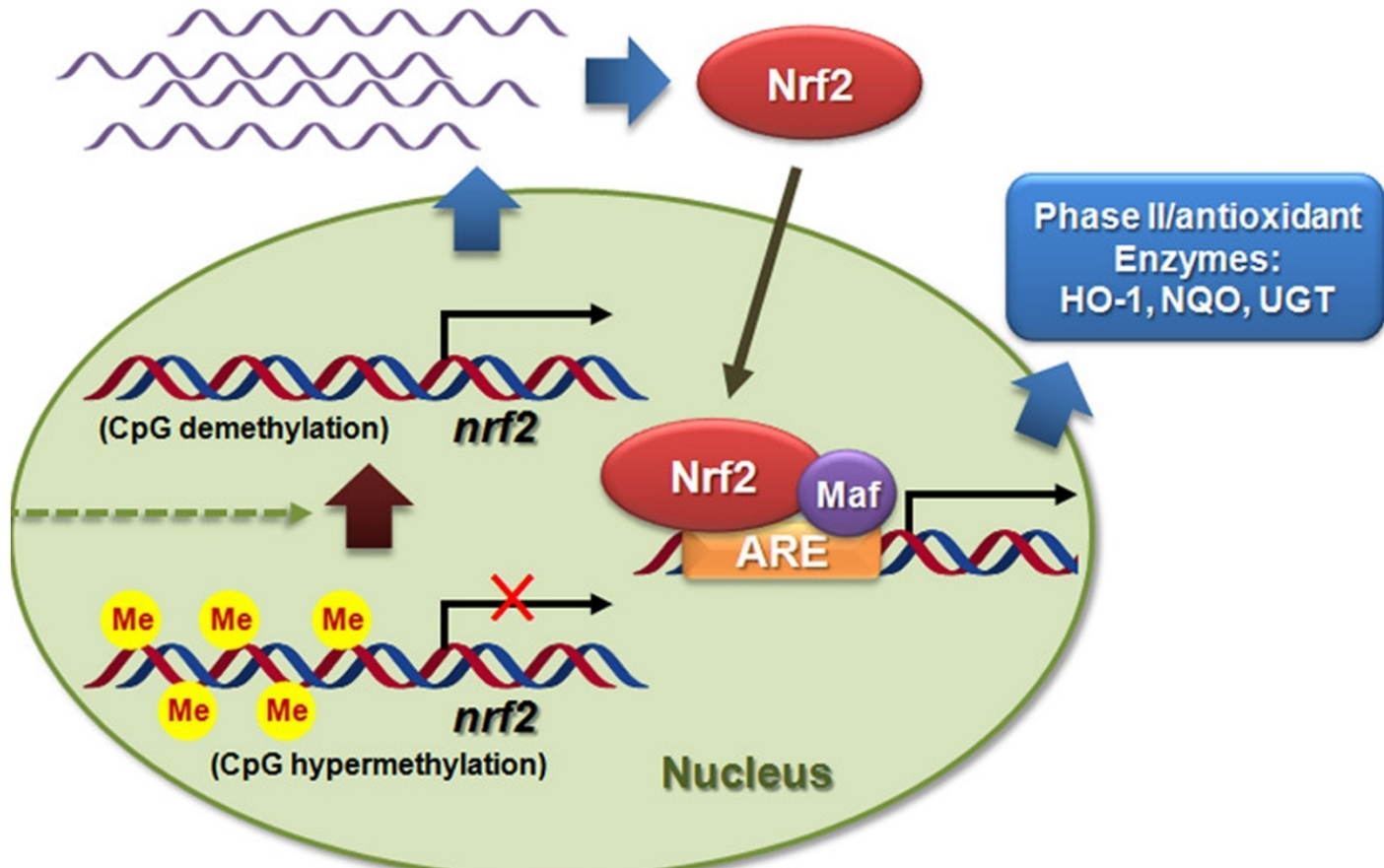








## Epigenetic regulation of *Nrf2*





## Research gap and objectives

Although there are intensive investigations on *Nrf2* however, little is known regarding the genetic and epigenetic regulation of bovine *Nrf2* signalling and its subsequent impacts on germinal cells

Microsatellite located in *Nrf2* gene may have potential influence on stress capacity of bovine sperm

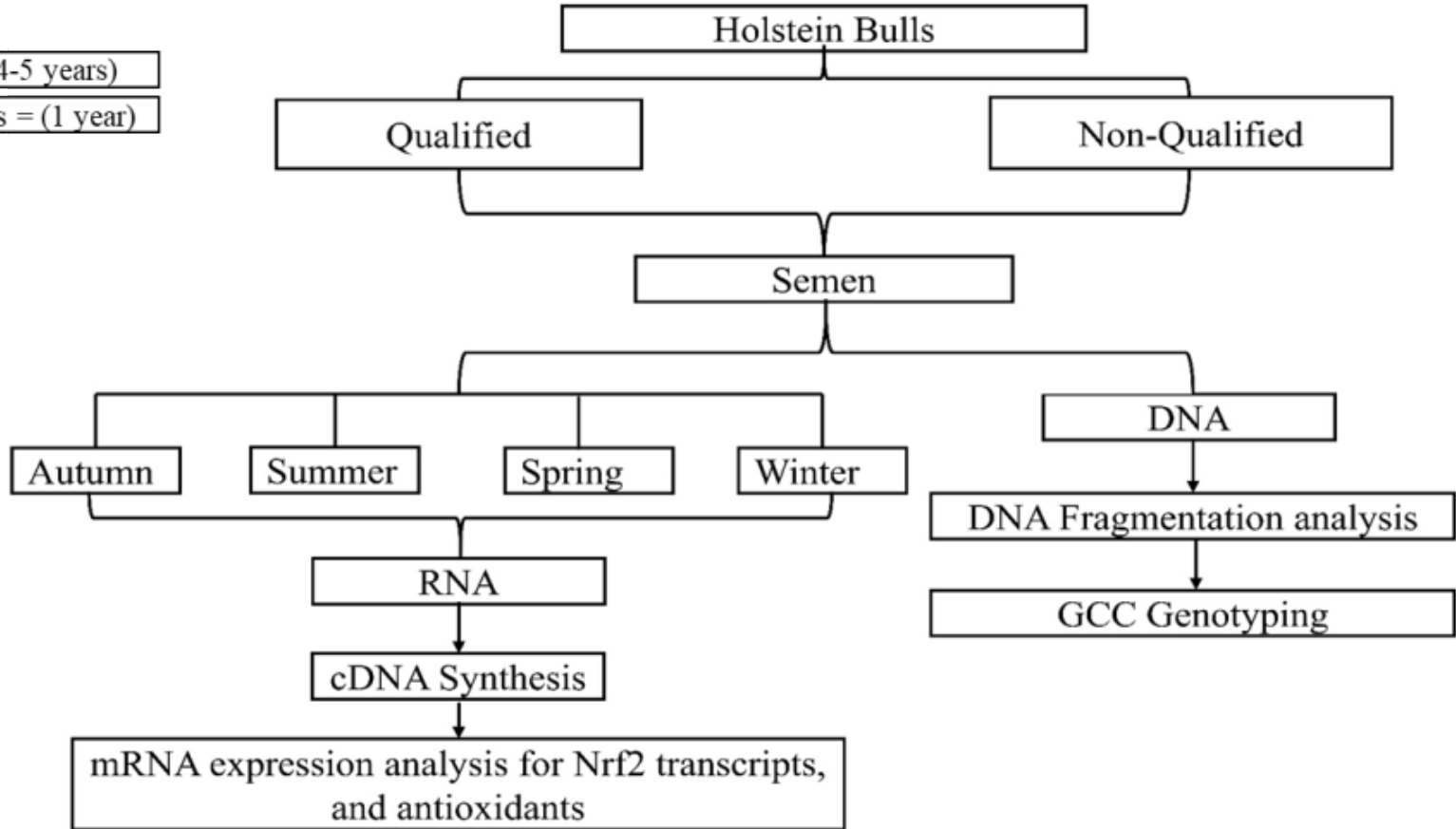
### The aim of this study is to investigate:

- The correlation between GCC microsatellite sequence in 1<sup>st</sup> exon of bovine *Nrf2* and the mRNA level of sperm-borne *Nrf2* and its downstream transcripts
- Association between sperm-borne antioxidant capacity and sperm quality under different conditions



# Experimental design

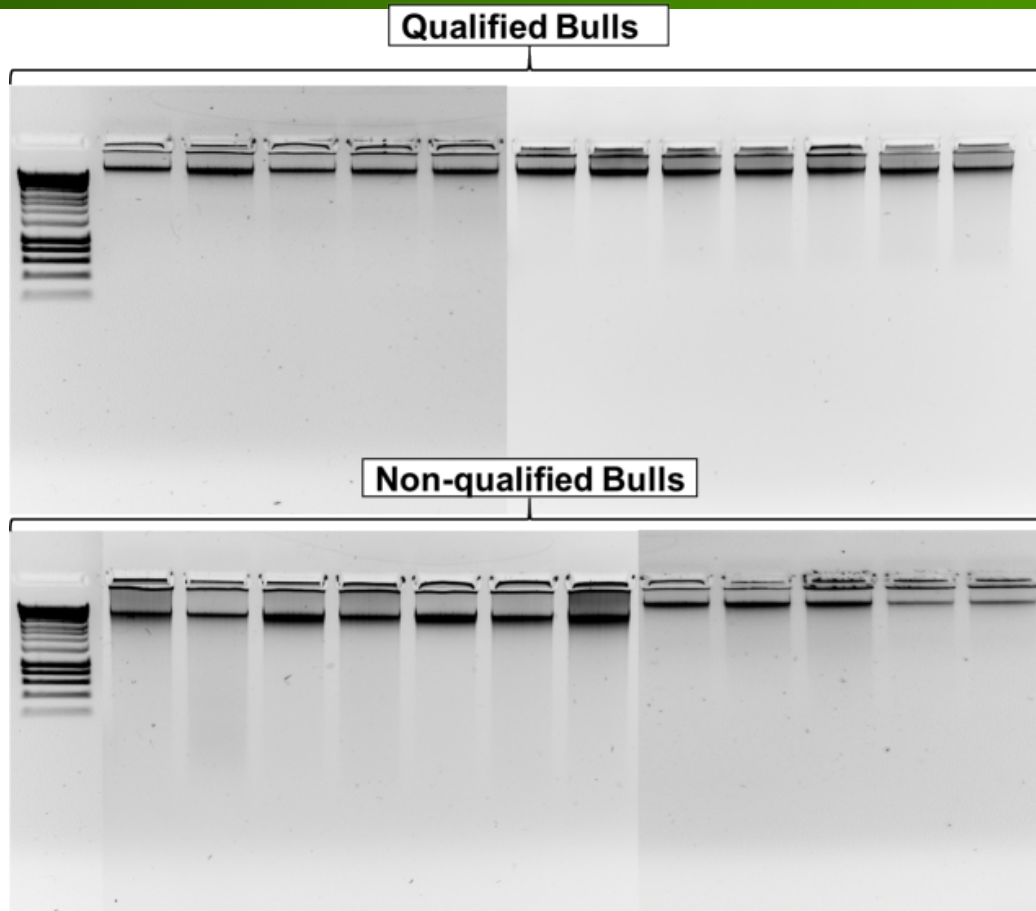
Qualified Bulls = (4-5 years)  
Non-qualified Bulls = (1 year)





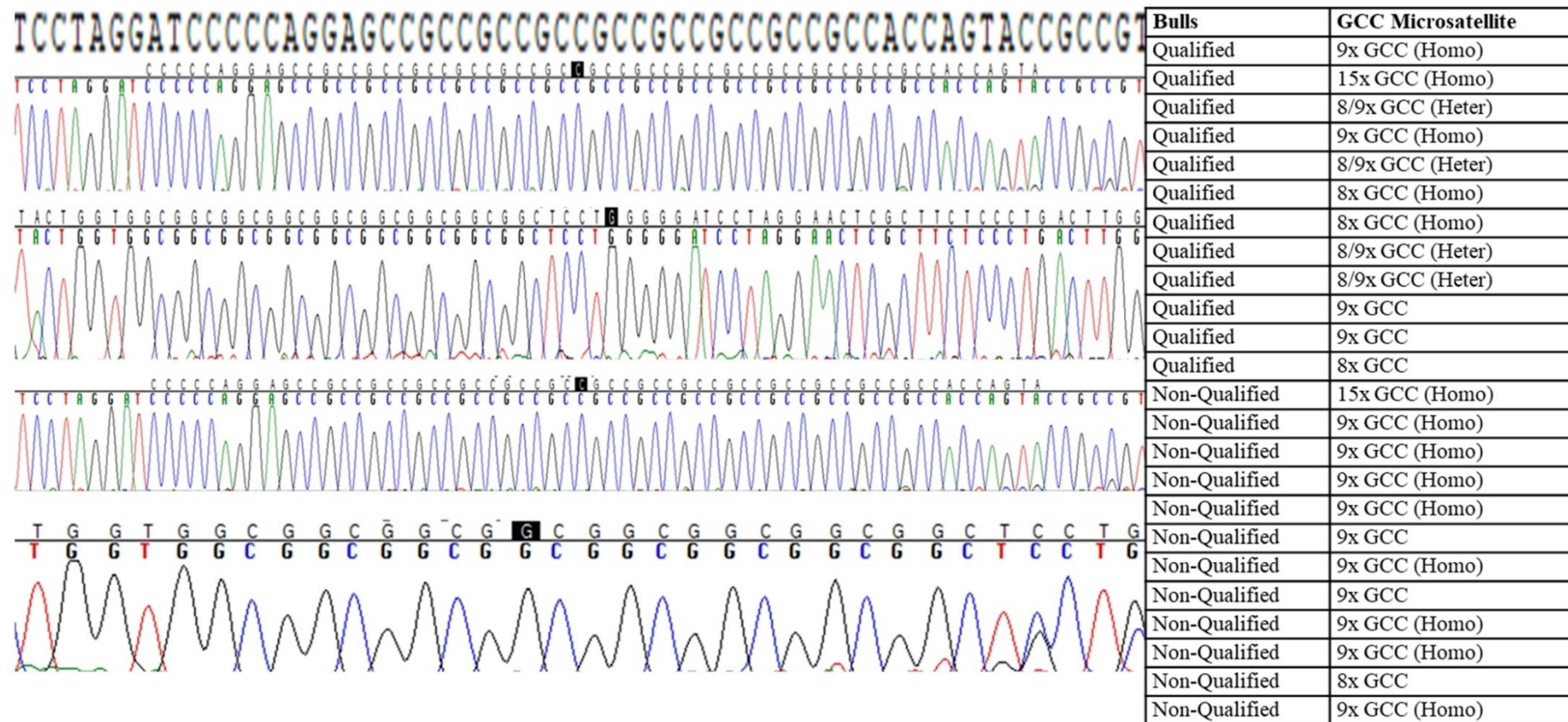


# Higher DNA fragmentation in sperm cells of non-qualified bulls



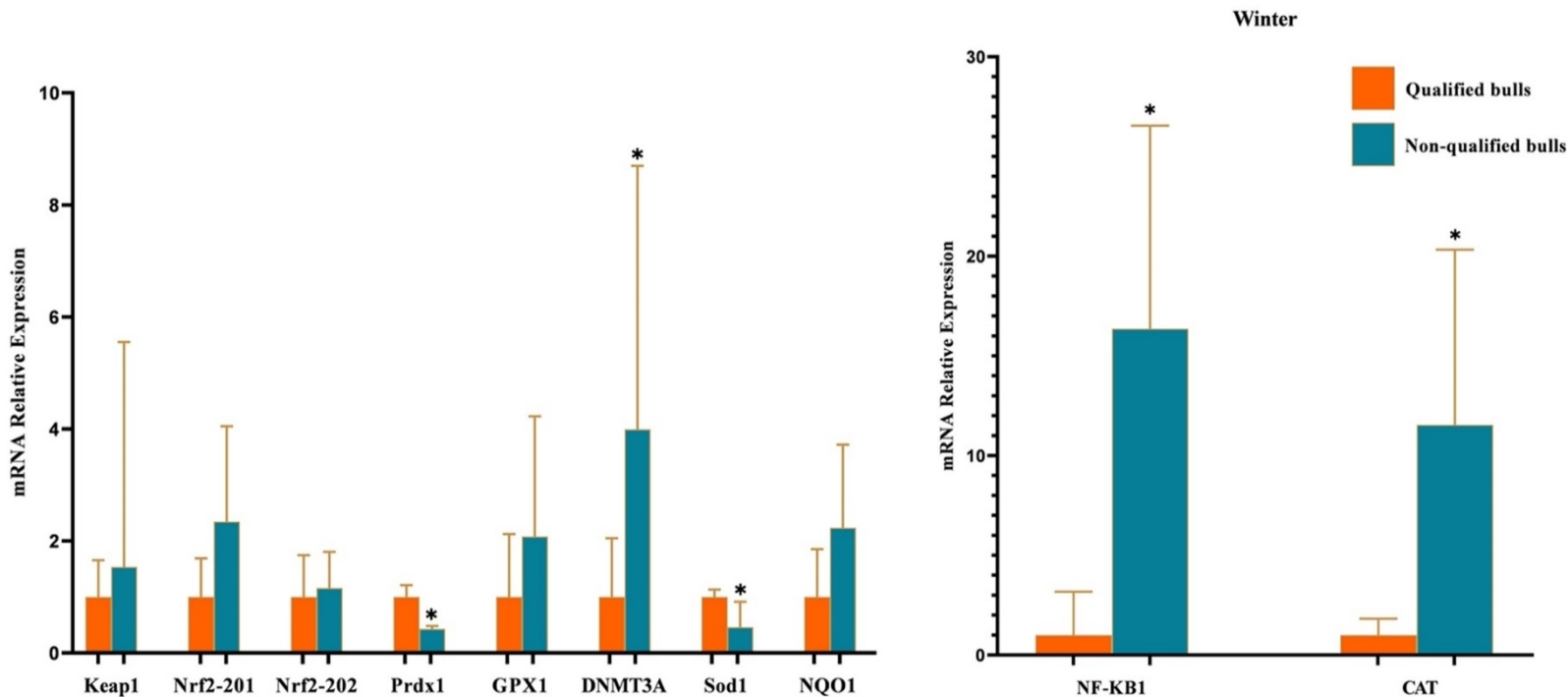


# Genotype analysis of *Nrf2* GCC microsatellite in spermatozoa DNA





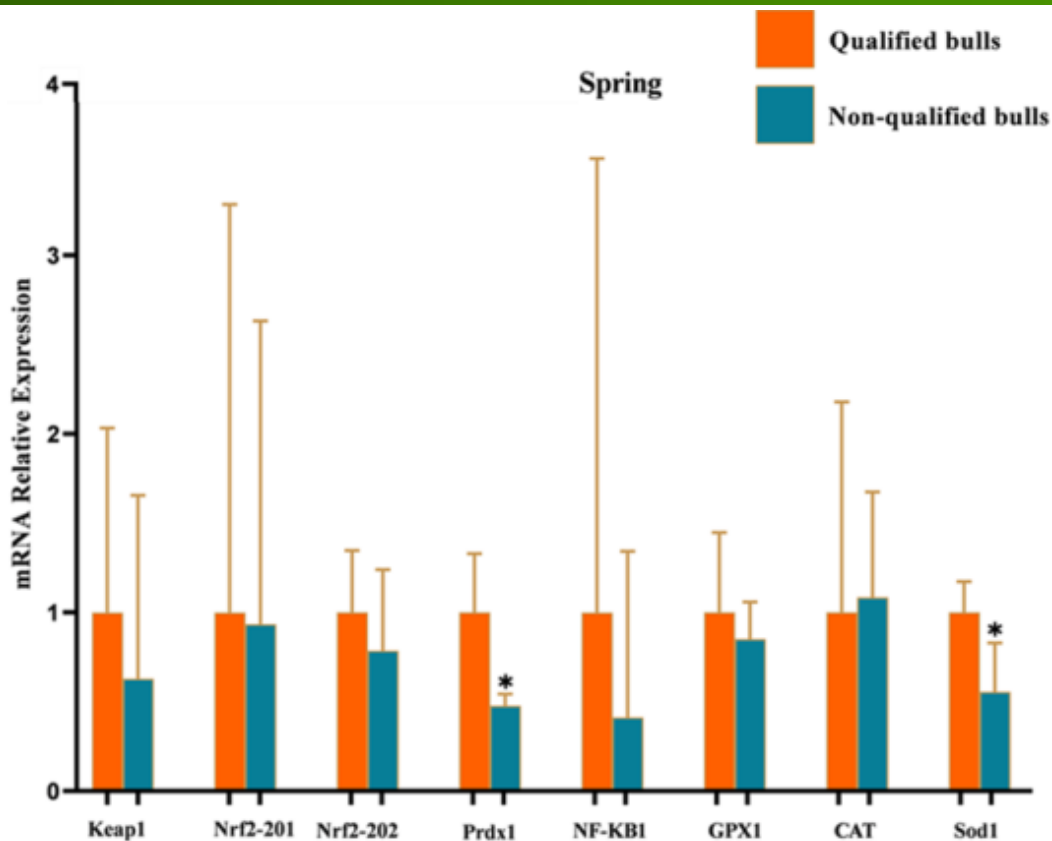
# Sperm-borne mRNA level of oxidative stress-related genes in



Keap1, Nuclear Factor Erythroid 2-Related Factor 2, Peroxiredoxin-1, Glutathione Peroxidase 1, DNA Methyltransferase 3 Alpha, Superoxide Dismutase 1, NAD(P)H Quinone Dehydrogenase 1, Nuclear Factor Kappa B Subunit 1, Catalase. Values are presented as geometric mean with geometric  $\pm$  SD. \* $p < 0.05$ , significant difference.



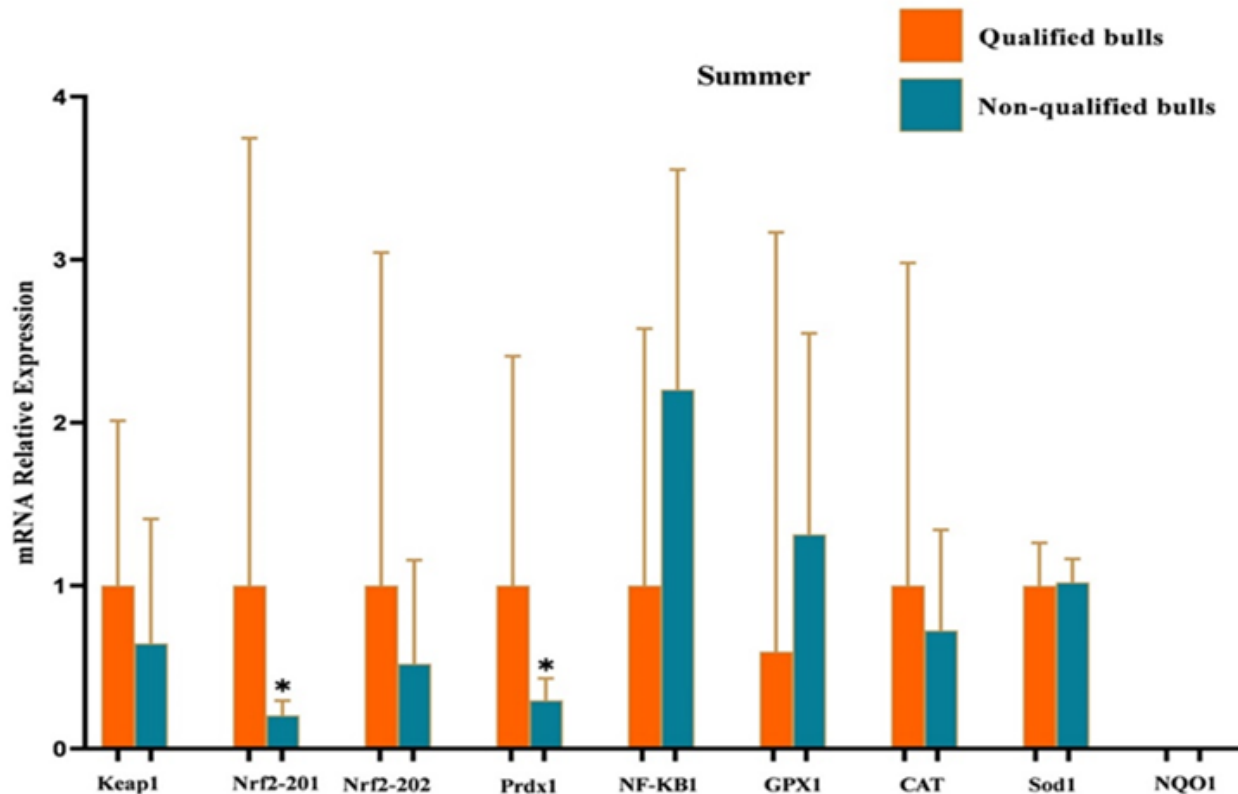
# Sperm-borne mRNA level of oxidative stress-related genes in Spring



Keap1 Like ECH Associated Protein 1, Nuclear Factor Erythroid 2-Related Factor 2, Peroxiredoxin-1, Nuclear Factor Kappa B Subunit 1, Glutathione Peroxidase 1, Catalase, Superoxide Dismutase 1. Values are presented as geometric mean with geometric  $\pm$  SD. \* $p < 0.05$ , significant difference.



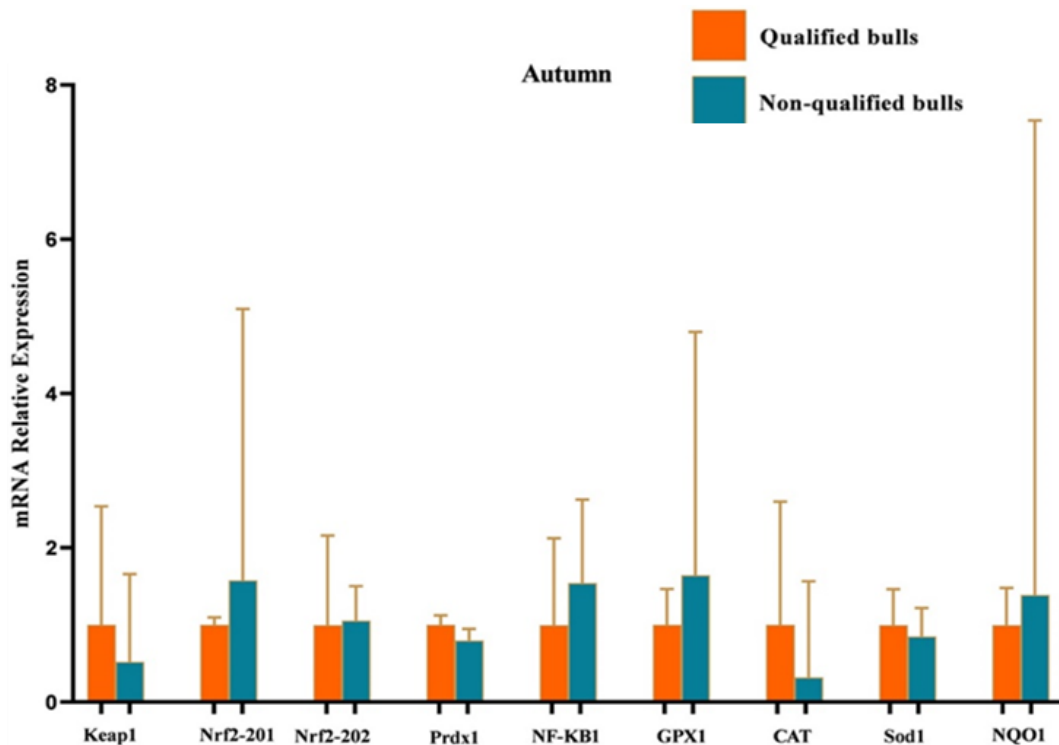
# Sperm-borne mRNA level of oxidative stress-related genes in Summer



*Kelch Like ECH Associated Protein 1, Nuclear Factor Erythroid 2-Related Factor 2, Peroxiredoxin-1, Nuclear Factor Kappa B Subunit 1, Glutathione Peroxidase 1, Catalase, Superoxide Dismutase 1, NAD(P)H Quinone Dehydrogenase 1.* Values are presented as geometric mean with geometric  $\pm$  SD. \* $p < 0.05$ , significant difference.



# Sperm-borne mRNA level of oxidative stress-related genes in Autumn

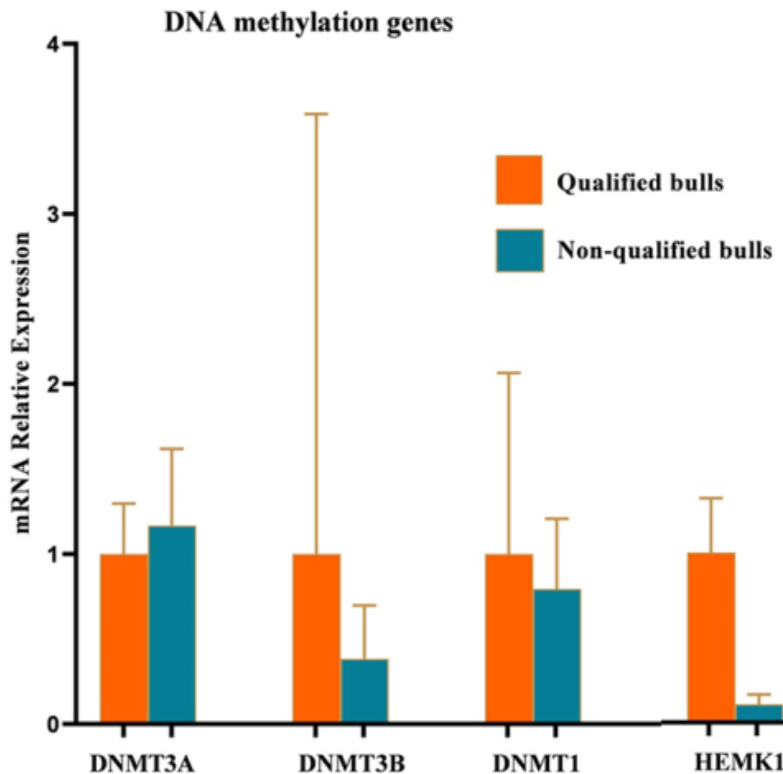


*Kelch Like ECH Associated Protein 1, Nuclear Factor Erythroid 2-Related Factor 2, Peroxiredoxin-1, Nuclear Factor Kappa B Subunit 1, Glutathione Peroxidase 1, Catalase, Superoxide Dismutase 1, NAD(P)H Quinone Dehydrogenase 1. Values are presented as geometric mean with geometric  $\pm$  SD.*





# Sperm-borne mRNA level of oxidative stress and methylation-related genes

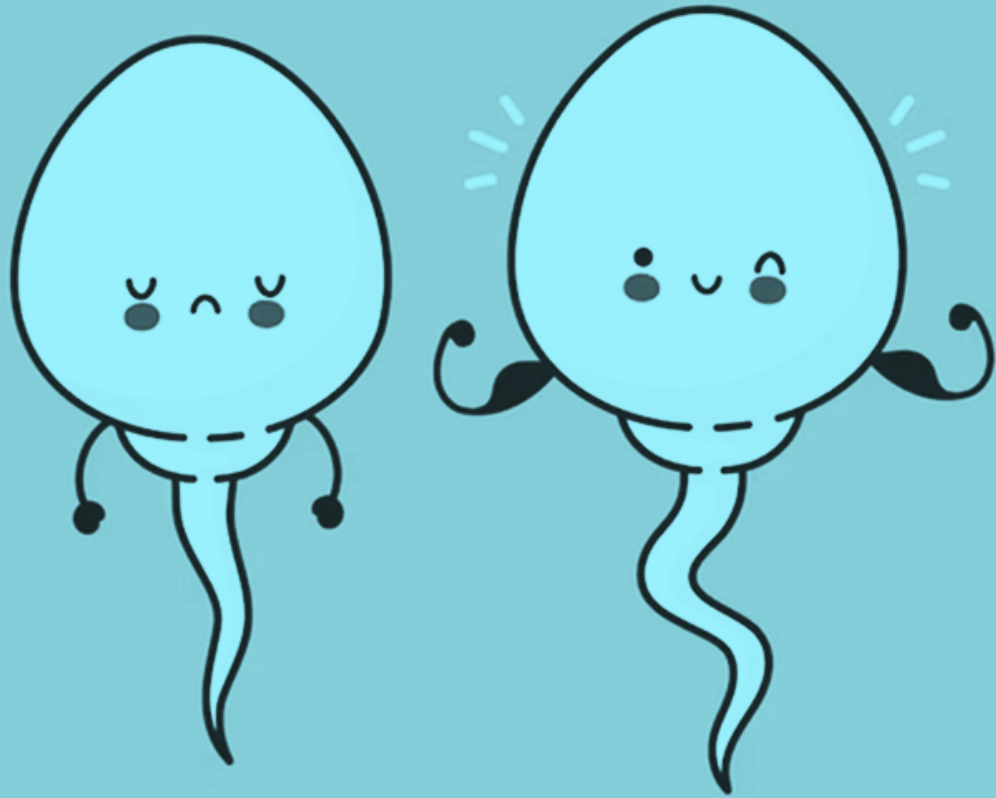


*DNA Methyltransferase 3 Alpha, DNA Methyltransferase 3 Beta, DNA Methyltransferase 1, HemK Methyltransferase Family Member 1.* Values are presented as geometric mean with geometric  $\pm$  SD.



## Conclusion & take-home message

- The significant higher expression of *Prdx1*, *Sod1*, and *Nrf2-201* in old bulls showed the multi-faceted nature of *Nrf2* which regulates a large variety of antioxidant genes including *Prdx1*, *Sod1*.
- Thus looking at our results *Prdx1*, *Sod1*, *Nrf2* and catalase regulated the oxidative stress during spermatogenesis in mature bulls.
- To fully understand the epigenetics of antioxidant system in gametes warrants further investigation.



*Thank You  
For Your  
Attention*