BovReg
Understanding cattle genomes

Effects of summer heat on in vitro fertility performance and possible epigenetic mechanisms of intergenerational transmission to offspring bulls

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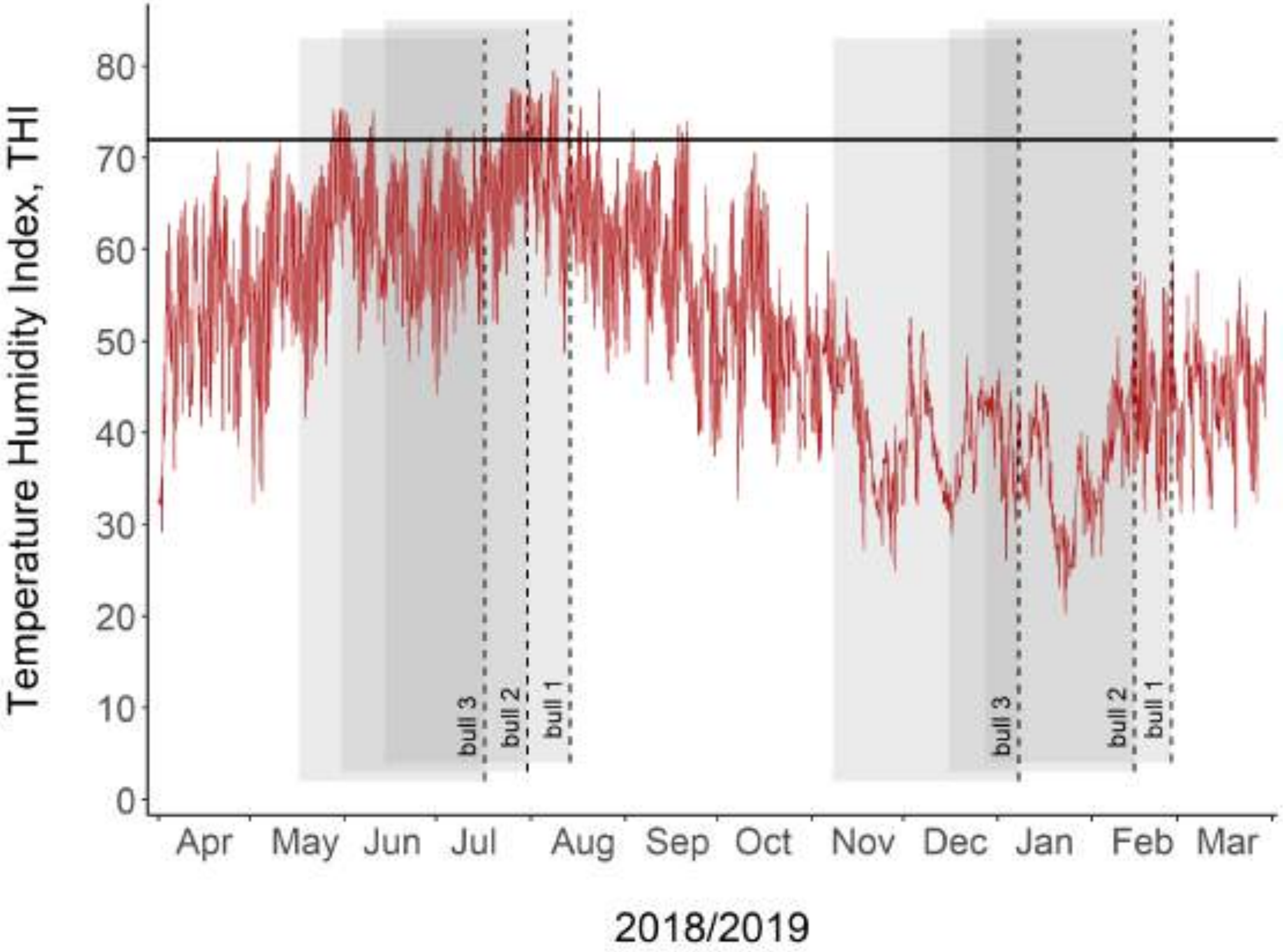
WP5: Epigenetics and environmental impact

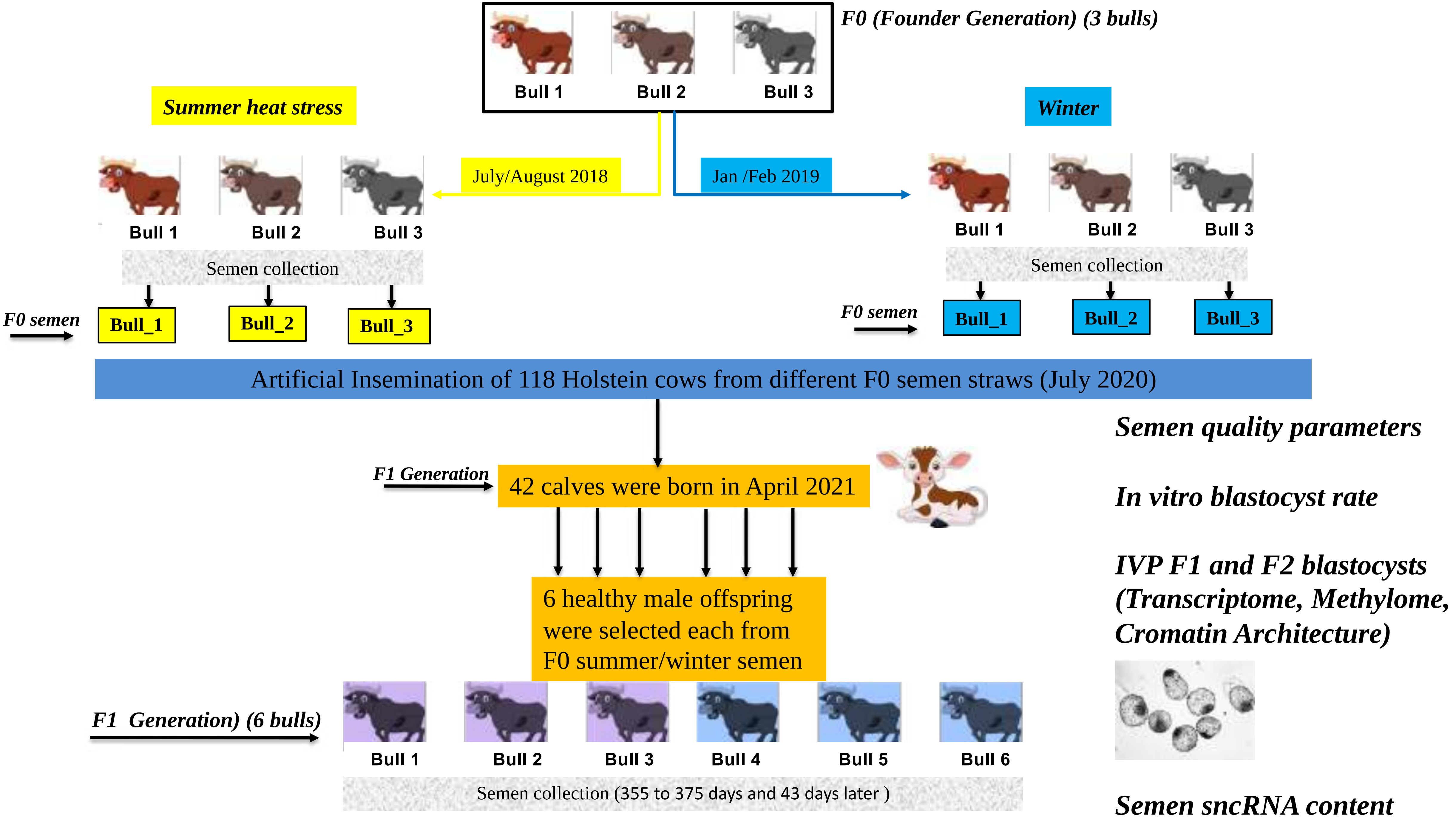
T5.3: Study of paternal inter- and transgenerational transmission of heat stress induced epigenetic modulation

Partner: LUKE, FBN, ULIEGE.

Experimental approach:



Summer 2018 was the hottest in the state Mecklenburg-Western Pomerania with an average temperature of **19.2°C** compared to the multi-year average value of **16.3°C** since measurements began in 1881



F0 (Founder Generation) (3 bulls)

Bull 1 **Bull 2** **Bull 3**

Summer heat stress

July/August 2018

Bull 1 **Bull 2** **Bull 3**

Semen collection

F0 semen

Bull_1 **Bull_2** **Bull_3**

Winter

Jan /Feb 2019

Bull 1 **Bull 2** **Bull 3**

Semen collection

F0 semen

Bull_1 **Bull_2** **Bull_3**

Artificial Insemination of 118 Holstein cows from different F0 semen straws (July 2020)

F1 Generation

42 calves were born in April 2021

6 healthy male offspring were selected each from F0 summer/winter semen

F1 Generation) (6 bulls)

Bull 1 **Bull 2** **Bull 3** **Bull 4** **Bull 5** **Bull 6**

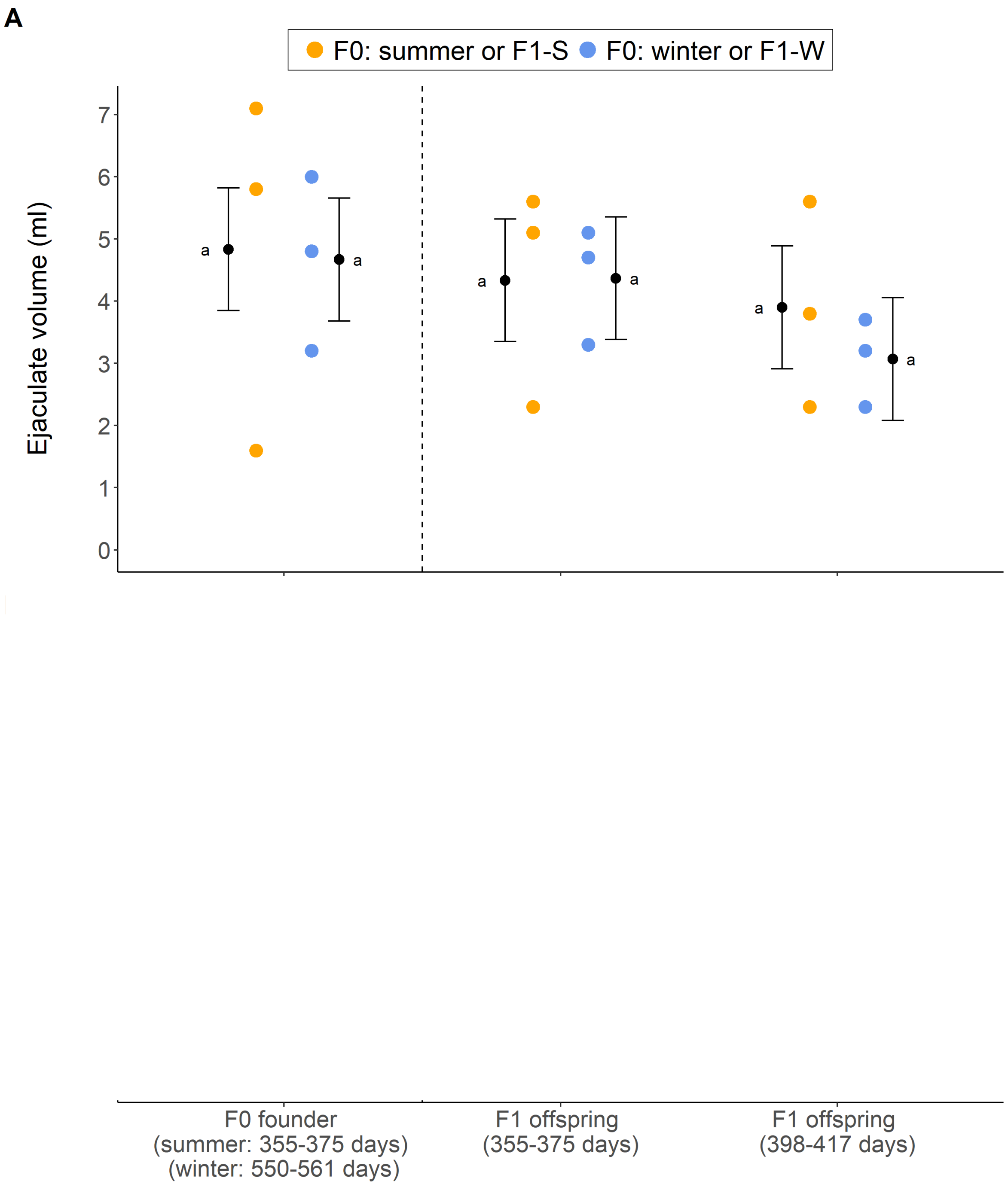
Semen collection (355 to 375 days and 43 days later)

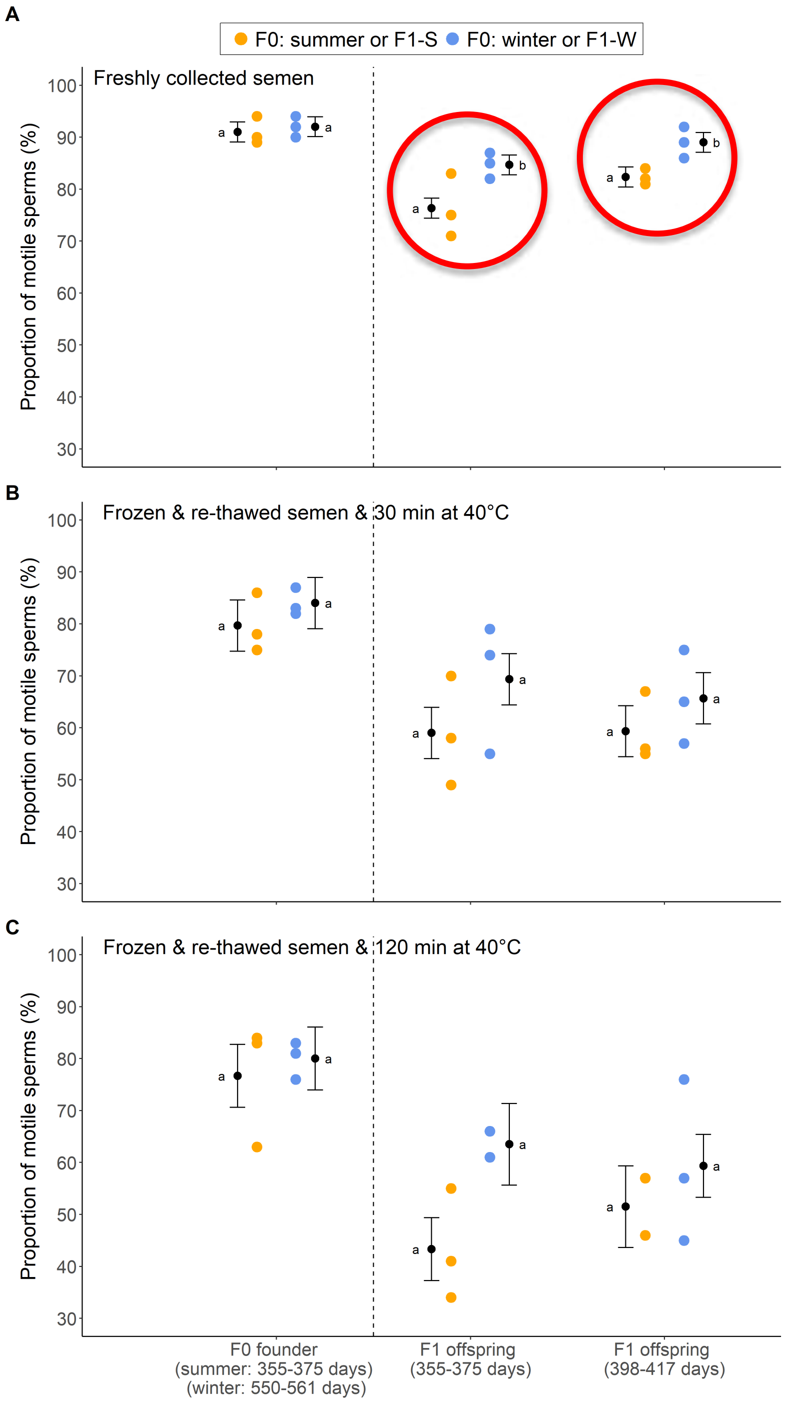
Semen quality parameters

In vitro blastocyst rate

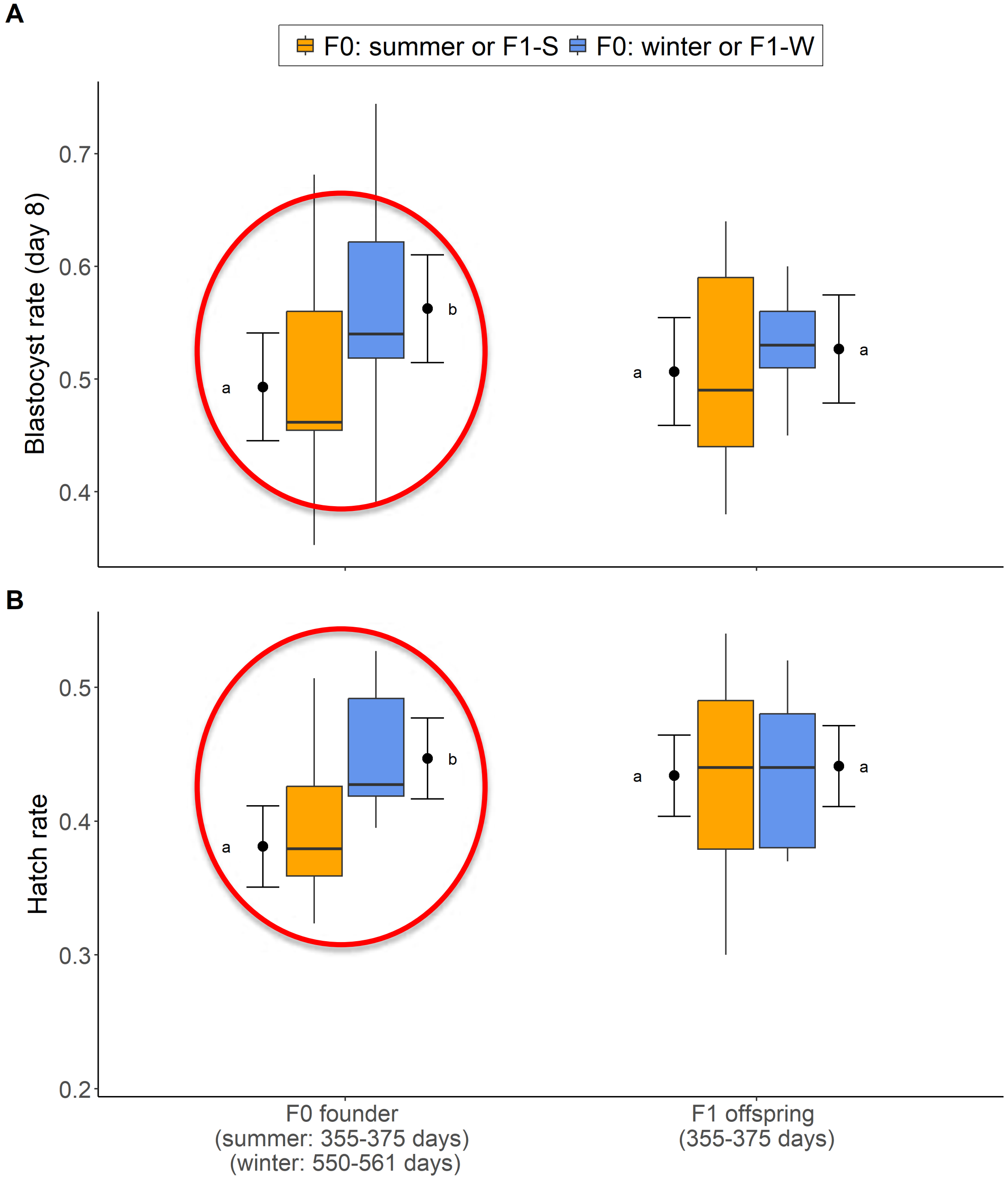
IVP F1 and F2 blastocysts (Transcriptome, Methylome, Cromatin Architecture)

Semen sncRNA content


A
F0: summer or F1-S
F0: winter or F1-W
Ejaculate volume (ml)
0
1
2
3
4
5
6
7
a
a
a
a
a
a
F0 founder
(summer: 355-375 days)
(winter: 550-561 days)
F1 offspring
(355-375 days)
F1 offspring
(398-417 days)


A
F0: summer or F1-S
F0: winter or F1-W
Freshly collected semen
Proportion of motile sperms (%)
30
40
50
60
70
80
90
100
a
a
a
b
a
b
B
Frozen & re-thawed semen & 30 min at 40°C
Proportion of motile sperms (%)
a
a
a
a
a
a
C
Frozen & re-thawed semen & 120 min at 40°C
Proportion of motile sperms (%)
a
a
a
a
a
a
F0 founder
(summer: 355-375 days)
(winter: 550-561 days)
F1 offspring
(355-375 days)
F1 offspring
(398-417 days)

S



Conclusions:

- Sperm motility of F1 sperm was affected.
- In vitro blastocyst/hatching rates of F0 sperm were affected.

Vanselow et al. 2024, provisionally accepted

Sperm sncRNA:
Possible vehicles of male epigenetic transmission?

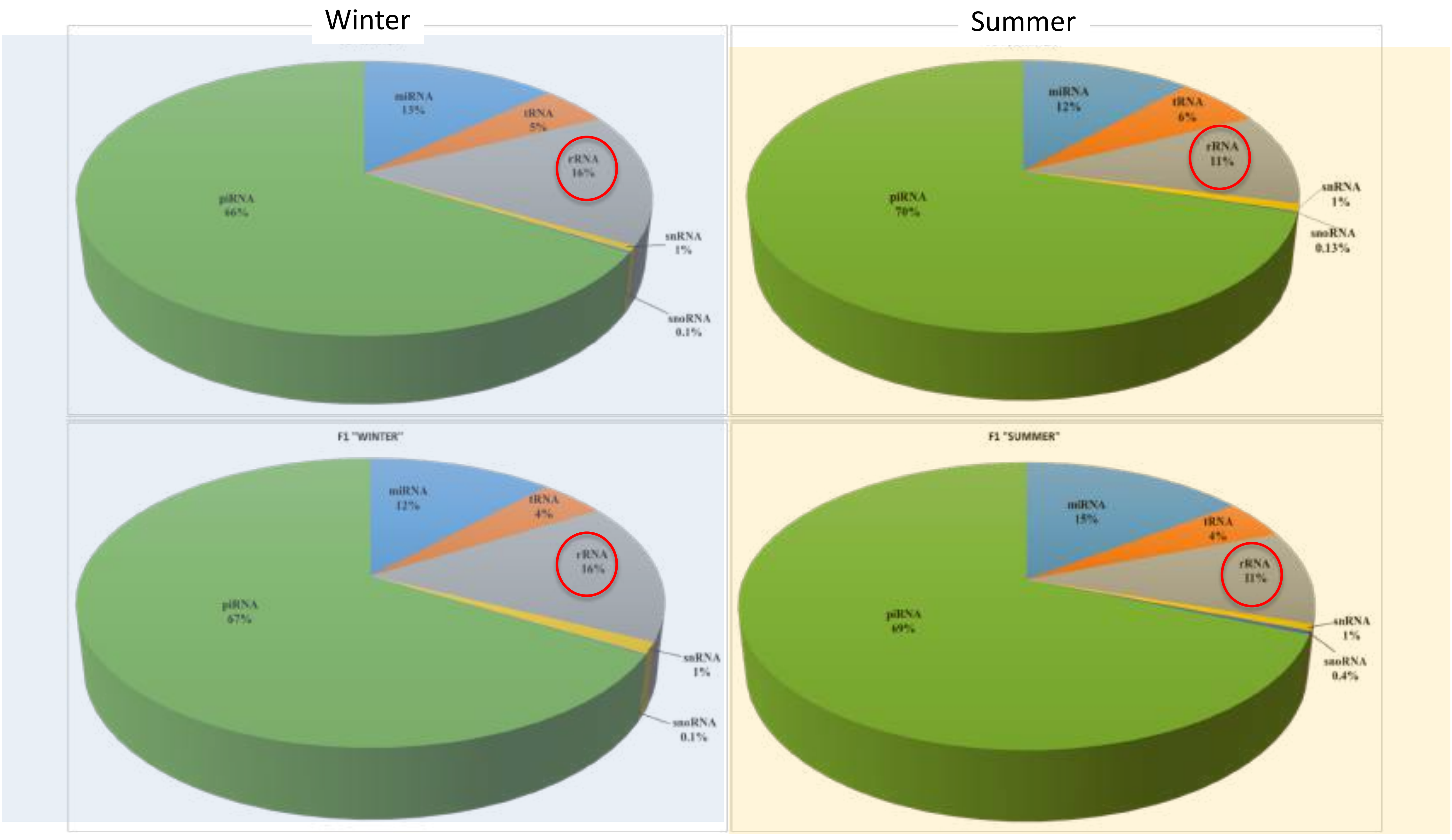
Experimental Approach:
Comparative analysis of F0 and F1 sperm sncRNA content

Winter

Summer

F0

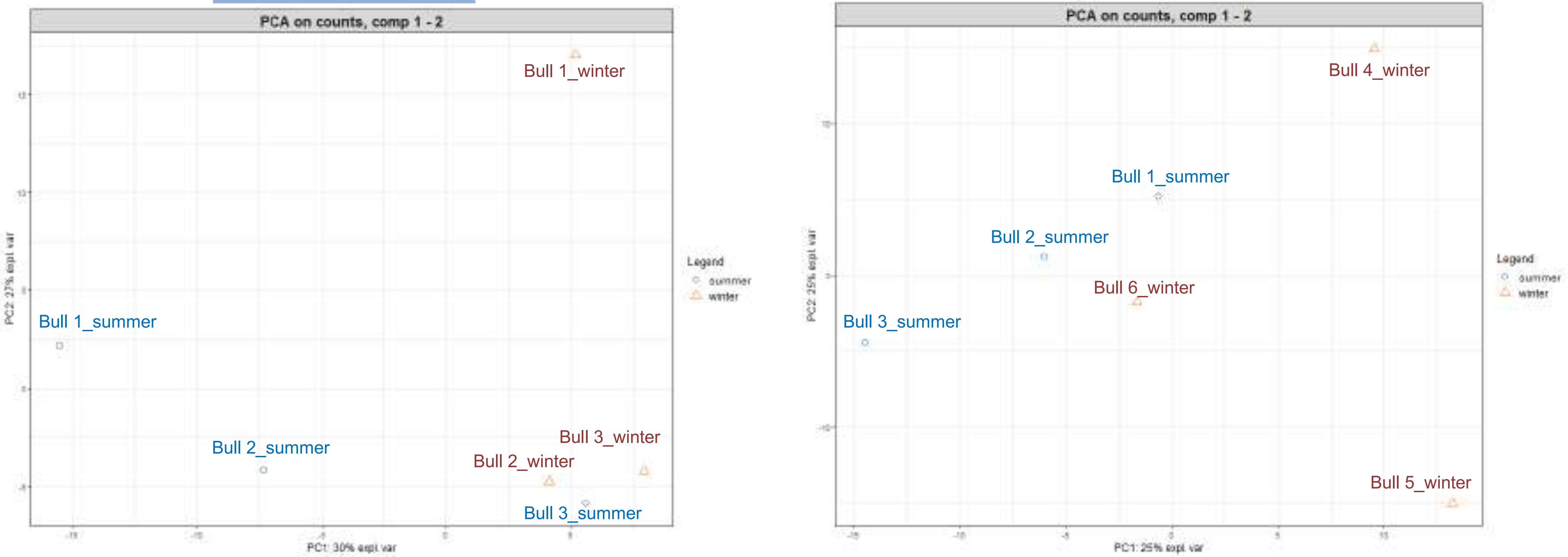
F1



Observations:

- Largest proportion (66-70%) of Piwi-interacting RNAs (piRNAs)
- Ribosomal RNA (rRNA) showed most differences winter vs. summer in F0 and F1 semen

miRNA data:



F0 bull generation

F1 bull generation

Principal component analysis (PCA) of miRNAs of the six F0 and the six F1 offspring semen samples

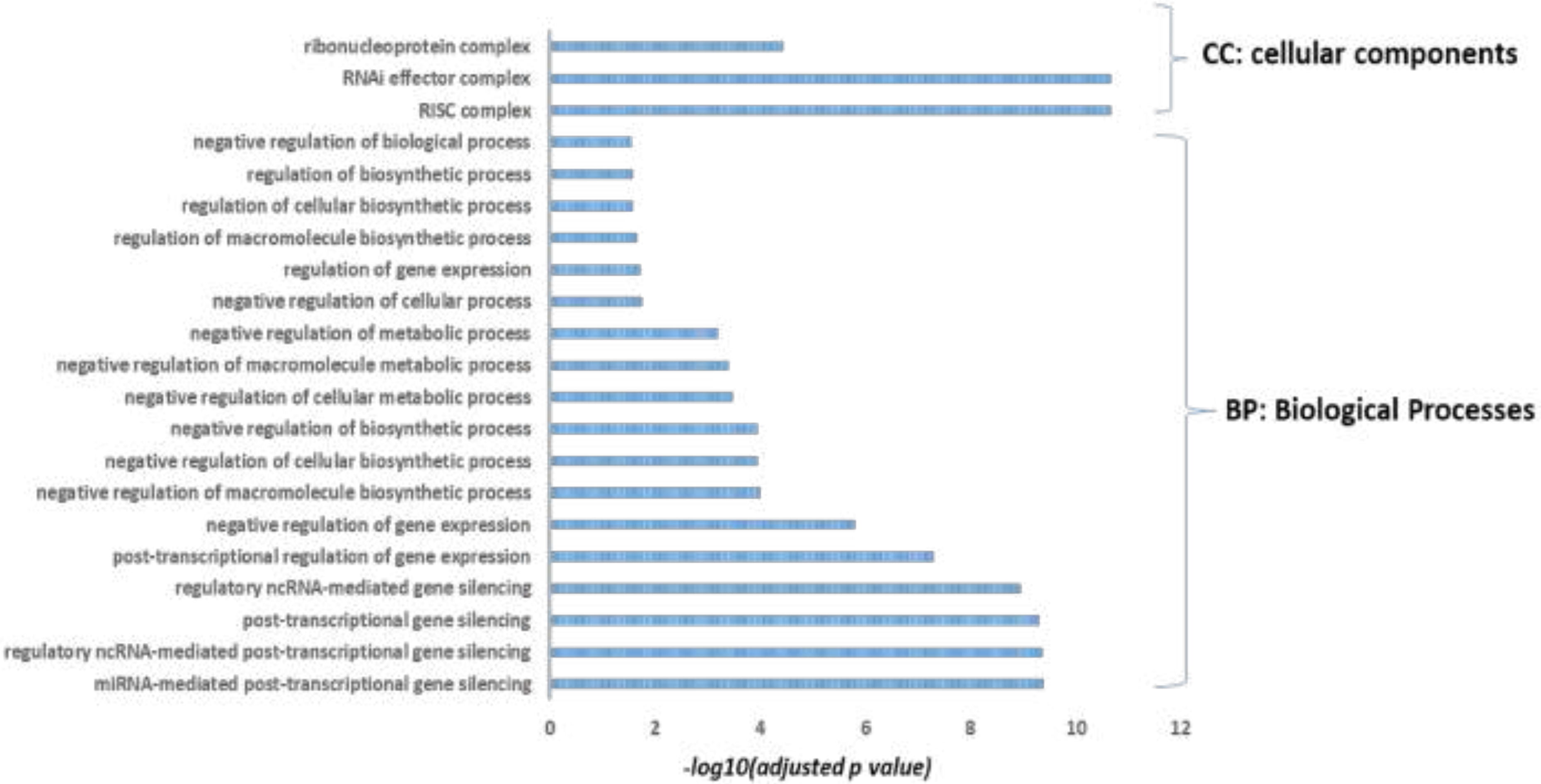
miRNA Results:

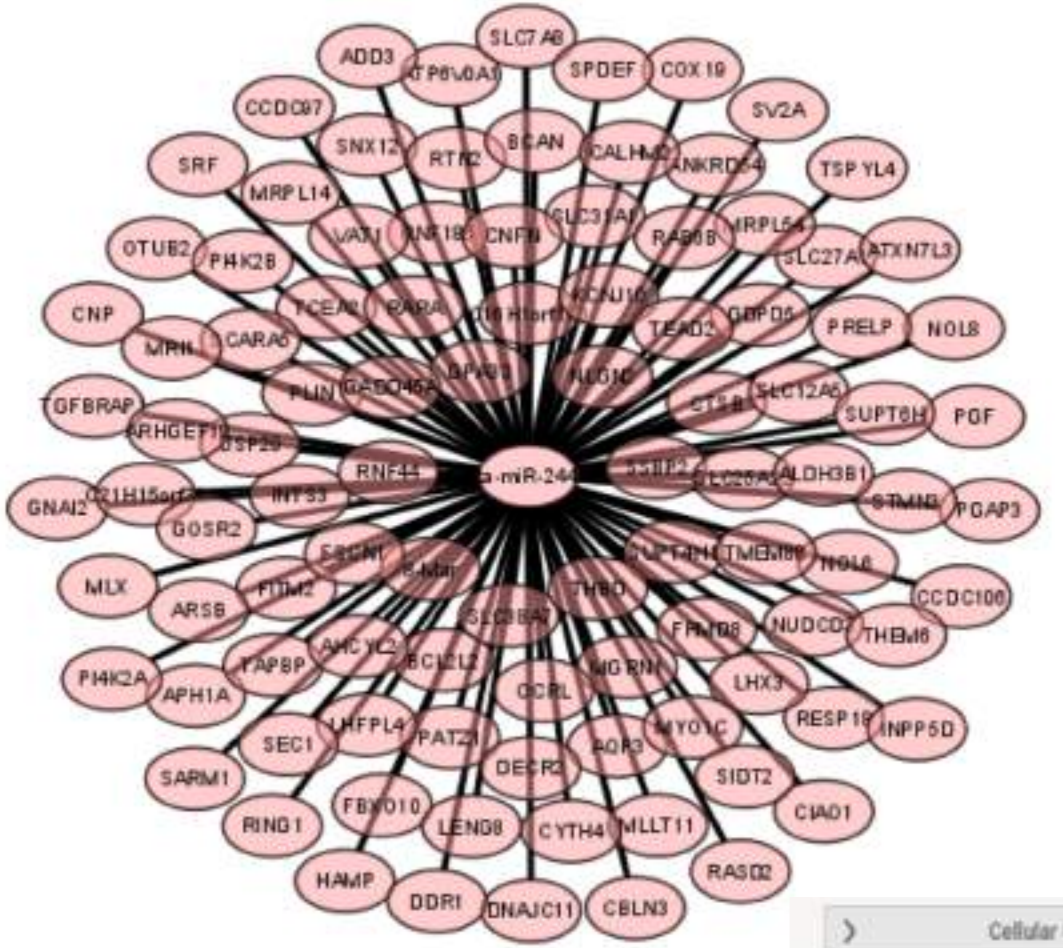
- No clear group-specific (summer vs. winter) clustering of miRNAs

- But significant differences amoung individual miRNAs

MicroRNAs	log2FoldChange	Up/down regulation	P value	adjusted p values	Bull Generation
bta-miR-20a	1.352377	up	0.002175	0.156072672	F0
bta-miR-18a	1.549066	up	0.016754	0.534270963	F0
bta-miR-2443	-21.8407	down	8.90E-15	8.51286E-13	F0
bta-miR-6529a	-1.10913	down	0.011965	0.490580448	F0
bta-miR-2285at	-1.17871	down	0.024052	0.587173446	F0
bta-miR-181c	-2.48945	down	0.037464	0.587173446	F0
bta-miR-345-5p	-6.79031	down	0.042301	0.587173446	F0
bta-miR-574	1.987358	up	0.003063	0.287412086	F1
bta-miR-10b	1.248898	up	0.00314	0.287412086	F1
bta-miR-205	1.410603	up	0.006116	0.287412086	F1
bta-miR-2285at	1.17168	up	0.006483	0.287412086	F1
bta-miR-98	1.151283	up	0.008376	0.30380426	F1
bta-miR-224	1.025267	up	0.012025	0.369076884	F1
bta-miR-1246	1.439433	up	0.014583	0.387913474	F1
bta-miR-10a	1.124103	up	0.020194	0.503577436	F1
bta-miR-369-5p	1.264836	up	0.026128	0.613229411	F1
bta-let-7b	1.031882	up	0.033773	0.641695626	F1
bta-miR-145	6.540741	up	0.041181	0.64829006	F1
bta-miR-2425-5p	6.465568	up	0.042244	0.64829006	F1
bta-miR-190a	11.58598	down	0.001417	0.287412086	F1

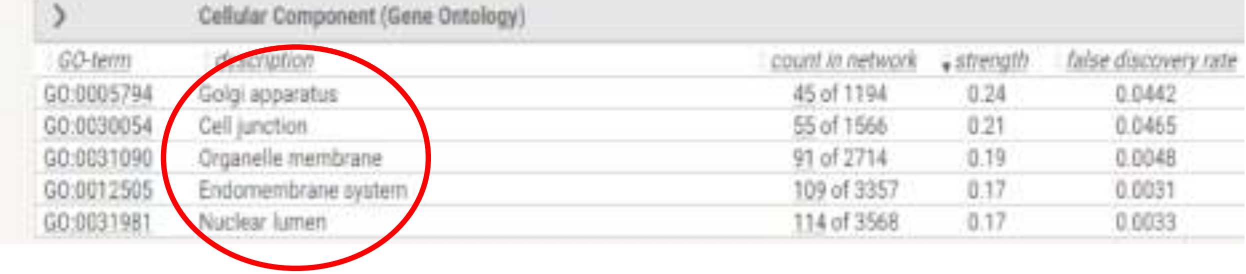
GO TERMS


ribonucleoprotein complex
RNAi effector complex
RISC complex
negative regulation of biological process
regulation of biosynthetic process
regulation of cellular biosynthetic process
regulation of macromolecule biosynthetic process
regulation of gene expression
negative regulation of cellular process
negative regulation of metabolic process
negative regulation of macromolecule metabolic process
negative regulation of cellular metabolic process
negative regulation of biosynthetic process
negative regulation of cellular biosynthetic process
negative regulation of macromolecule biosynthetic process
negative regulation of gene expression
post-transcriptional regulation of gene expression
regulatory ncRNA-mediated gene silencing
post-transcriptional gene silencing
regulatory ncRNA-mediated post-transcriptional gene silencing
miRNA-mediated post-transcriptional gene silencing
0
2
4
6
8
10
12
-log10(adjusted p value)
CC: cellular components
BP: Biological Processes



99 of 2065 predicted genes regulated by miRNAs *bta-miR-2443* that was found highly down-regulated in summer heat stressed semen samples (level of significance as log2foldchange of -21 and *p* value = 8.90E-15).

Top 500 targeted by *bta-miR-2443* belonged to major cellular functions

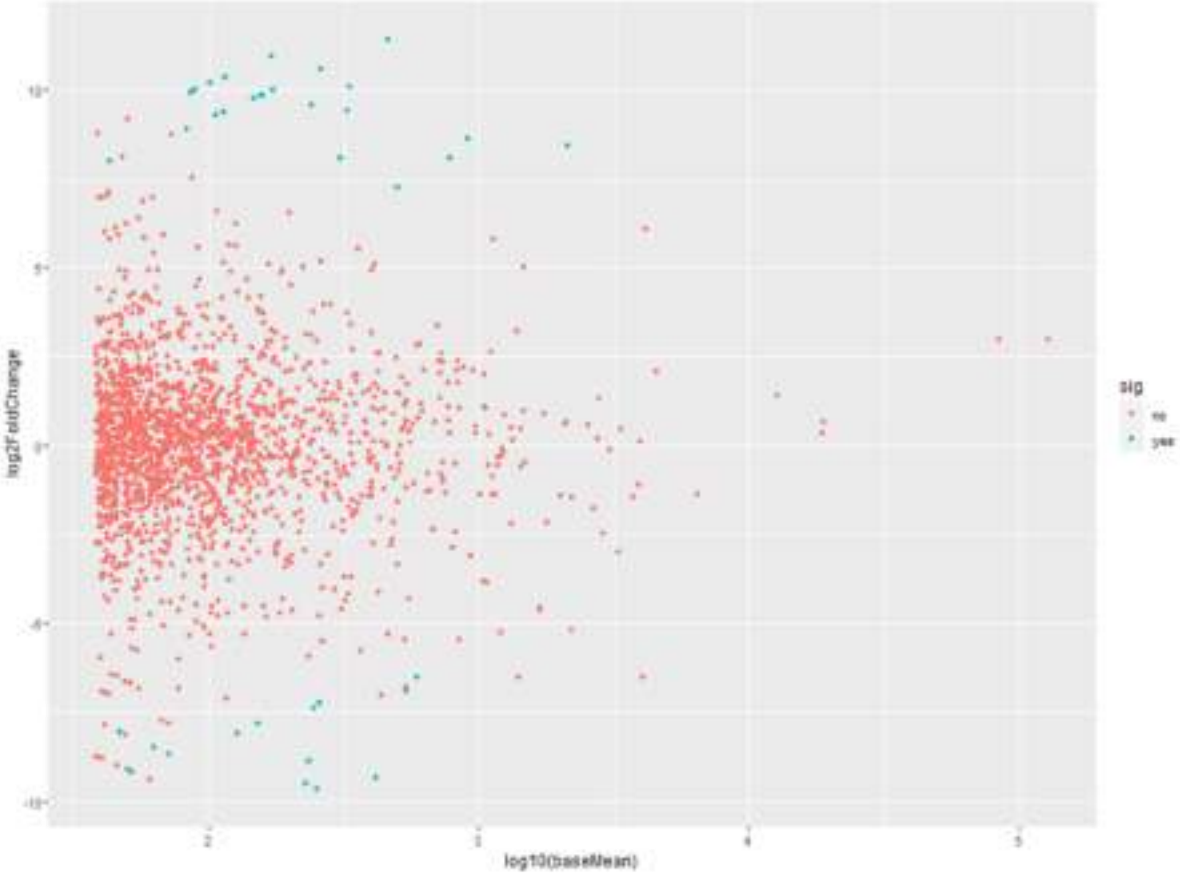


Cellular Component (Gene Ontology)

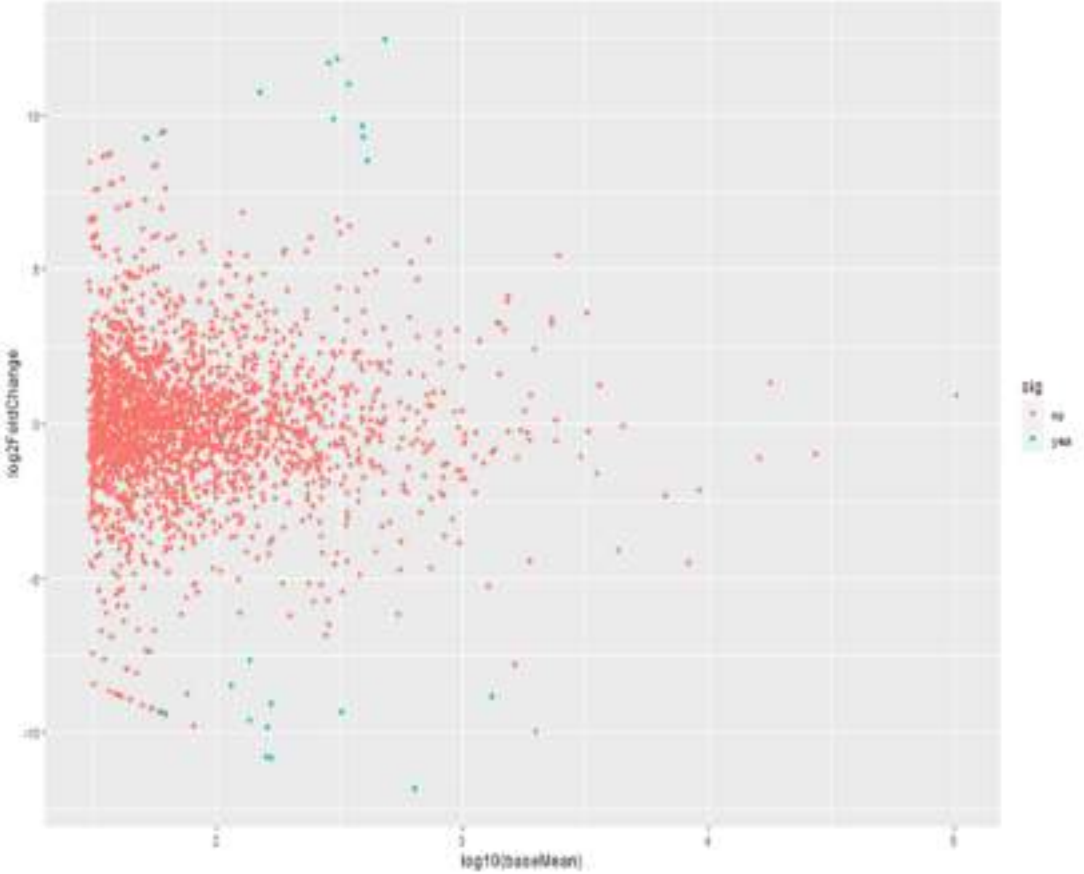
GO-term	description	count in network	strength	false discovery rate
GO:0005794	Golgi apparatus	45 of 1194	0.24	0.0442
GO:0030054	Cell junction	55 of 1566	0.21	0.0465
GO:0031090	Organelle membrane	91 of 2714	0.19	0.0048
GO:0012505	Endomembrane system	109 of 3357	0.17	0.0031
GO:0031981	Nuclear lumen	114 of 3568	0.17	0.0033

rRNA data:

F0 bull generation



F1 bull generation



F0 semen: 37 differentially expressed rRNAs (23 up and 14 down-regulated)

F1 semen: 23 differentially expressed rRNAs (11 up and 12 down-regulated)

Conclusions:

- Natural summer heat stress during spermatogenesis affected in vitro blastocyst rates.
- Motility was affected in semen samples from F1 offspring (transgenerational!).

Hypothesis:

- Heat effects on semen quality parameters are transmitted possibly via sncRNA (rRNA?) to the next generation.

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