FACULTY OF VETERINARY MEDICINE
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EXPOSURE OF BULLS TO HEAT STRESS HAD DELETERIOUS EFFECTS ON EMBRYO DEVELOPMENT

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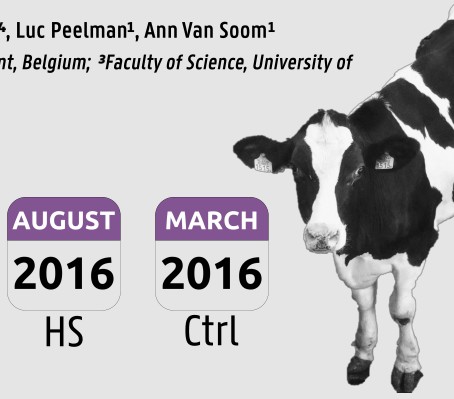
At present, breeding companies are having concerns about the possible impact of natural heat stress on animal fertility. The goal of our project is to determine the effects of increased environmental temperature on Holstein bulls and its effects on sperm quality and embryo development.

Experimental design

Frozen bovine semen samples were obtained from 6 Holstein bulls exposed on 3 consecutive days to natural heat stress (HS) (August 2016, max. THI 74.5), and to lower temperature (Ctrl) (March 2016, max. THI 40.6).

THI: Temperature-Humidity Index



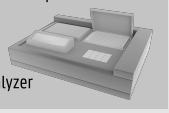
Methodology

- Embryo development rates: *In Vitro* Fertilization (IVF)

- Sperm morphology: eosin/nigrosin staining
- Day-8 blastocysts Inner cell mass/trophectoderm ratio (ICM/TE), and apoptosis cell ratio : Differential staining

*Optical / confocal microscopy

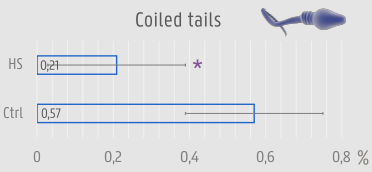
- Day-8 blastocysts gene expression: Real-time quantitative polymerase chain reaction (RT-qPCR)

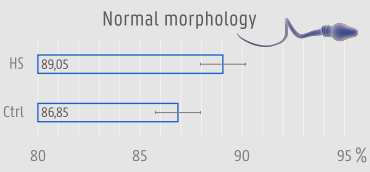


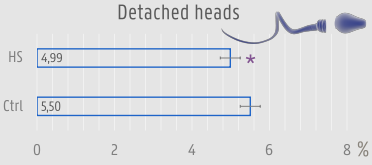
*RT-qPCR analyzer

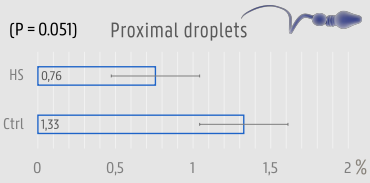
Results

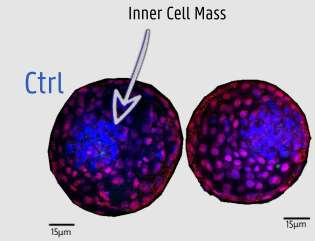
* Indicates p<0.05

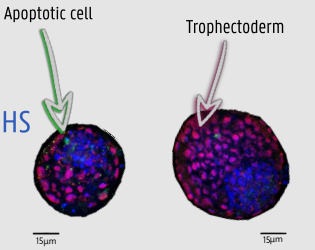


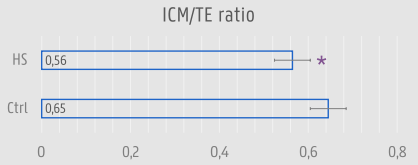


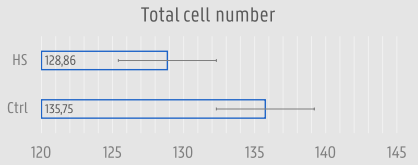


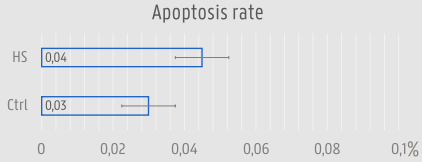






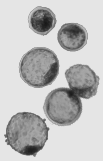


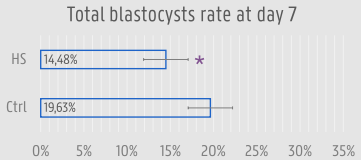


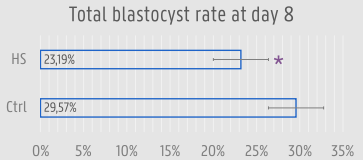


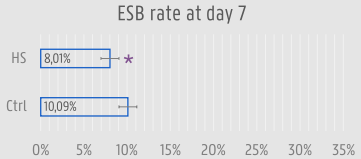
Imprinted genes			Heat-Shock Protein genes			Pluripotency genes		
Gene	P value	HS/Ctrl ratio	Gene	P value	HS/Ctrl ratio	Gene	P value	HS/Ctrl ratio
H19	0.375	4.146	HSPA1A	0.825	1.272	SOX2	0.625	1.553
IGF2	0.625	2.426	HSPA2	0.375	2.536	NANOG	0.750	1.102
MEST	0.825	1.591	HSPA8	1.000	0.931	POU5F1	0.825	1.104
MEG9	0.825	1.263	HSP10	0.825	0.874			
MEG3	0.825	1.314	HSP60	0.825	1.301			
SNRPN	0.825	1.035	HSP90	0.825	1.145	**DNMT genes**		
PHLDA2	0.825	0.869	HSF1	0.500	0.611	Gene	P value	HS/Ctrl ratio
PLAGL	0.964	1.138	**Interferon tau gene**			DNMT1	0.825	1.213
IGF2R	1.000	1.034	Gene	P value	HS/Ctrl ratio	DNMT3A	0.825	0.953
PEG10	1.000	1.224	IFNT2	0.625	1.608	DNMT3B	0.825	0.977

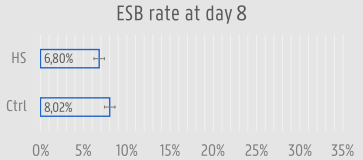
Wilcoxon signed rank 1: 6 pairs
Reference genes: ACTB, GAPD, SDHA

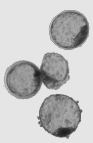


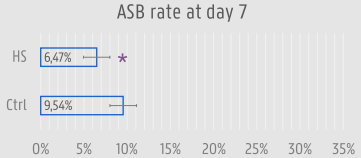


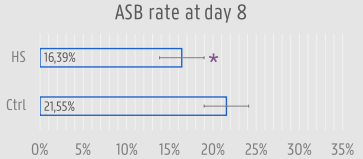












Early stage blastocysts (ESB): Early and Normal
Advanced stage blastocysts (ASB): Expanded, Hatching and Hatched

Conclusions

- Sperm samples collected in August had reduced fertility compared to those obtained in March.
- Although fewer sperm abnormalities were present in HS, based on decreased blastocyst rates and ICM/TE ratio in embryos produced with HS semen, we inferred that molecular mechanisms for advanced blastocyst development were affected.
- Those mechanisms did not involve our target genes.

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