HEAT STRESS EFFECTS ON REACTIVE OXYGEN SPECIES PRODUCTION AND LIPID PEROXIDATION IN BOVINE SPERMATOZOA

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Introduction

Heat-stressed semen displays:

- ❑Lower protamination
- ❑Lower sperm motility
- ❑Changes in the methylation of paternal pronuclei

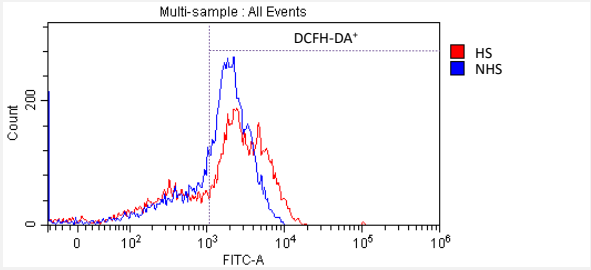
(Rahman et al., Theriogenology, 76, 1246–1257, 2011).

Objectives

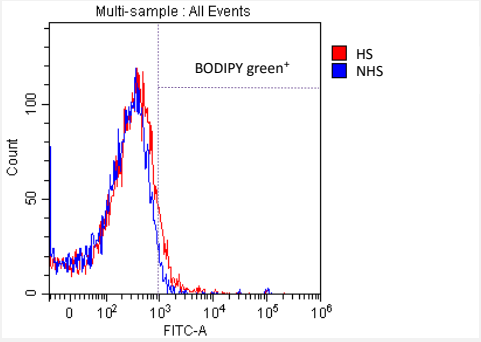
- ❑To elucidate the effects of heat stress on oxidative status in bovine spermatozoa by quantifying reactive oxygen species (ROS) and lipid peroxidation (LPO).
  - Heat-stressed (HS) and non-heat-stressed (NHS) frozen semen samples of Holstein-Friesian bulls

Flow cytometry

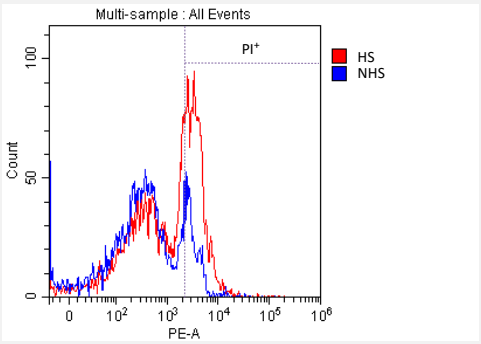
❑DCFH-DA → intracellular H_2O_2



❑BODIPY™ 581/591 C11 → peroxidized lipids



❑Propidium Iodide (PI) → membrane damage



Sperm stainings

- ❑ Luminol → ROS production
- ❑ Propidium Iodide (PI) → membrane damage
- ❑ DCFH-DA → intracellular H_2O_2
- ❑ BODIPY™ 581/591 C11 → peroxidized lipids

Results

	Heat Stress	No Heat Stress	P	N
PI - Membrane damage	30.69 ± 1.46%	21.89 ± 0.44%	0.0006	N=4
DCFH - Intracellular H_2O_2	34.79 ± 8.40%	35.31 ± 12.11%	0.9729	N=4
BODIPY – RED - Lipids	83.62 ± 0.92%	91.30 ± 1.57%	0.0057	N=4
BODIPY – GREEN - Oxidized lipids	5.84 ± 3.36%	3.13 ± 1.87%	0.3671	N=4
LUMINOL 15 MIN - ROS production	0.56 ± 0.24	0.38 ± 0.27	0.653	N=3
LUMINOL 30 MIN - ROS production	1.04 ± 0.45	0.79 ± 0.46	0.724	N=3

Data was analyzed using the Student t test (p≤0.05).

	Bodipy Green
Luminol	R=0.828
	P=0.04

Data was analyzed using correlation of Spearman (p≤0.05).

**Please ask the representing author for further experimental details*

Discussion and Conclusions

- No differences were observed in the percentage of DCFH-DA+ cells between HS and NHS semen. However, a higher mean fluorescence intensity (MFI) was observed in HS compared to NHS semen, indicating that HS cells have more intracellular H_2O_2.
- A positive correlation was observed between ROS production (luminol) and LPO (BODIPY green) (r=0.82, p=0.01).
- The survival rate of sperm cells was higher in NHS than in HS semen, while a higher LPO and ROS production were observed in HS compared to NHS semen. These results suggest a possible effect of heat stress on the oxidative status of bovine spermatozoa.

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