4 plains bison were collected by electro-ejaculation during the summer of 2007. Andrological parameters of morphology and motility were recorded on fresh semen, extended semen, and post-thawed semen samples. A Student's *t*-test was used to compare the results of these two groups. Semen was cryopreserved using two commercially available cryopreservation media (Andromed and Triladyl, Minitube Canada, Ingersoll, Ontario, Canada). Sperm morphology and motility were not different between electro-ejaculated samples from plains and wood bison ($P > 0.05$). Also, no difference was found in the survival rate of sperm from the electro-ejaculated samples between plains and wood bison after freezing and then thawing using an egg-yolk based extender (Triladyl) or an extender containing no products of animal origin (Andromed). A difference between cryopreservation media was found; post-thaw motility of Triladyl-treated sperm was higher (29%) than that of the Andromed-treated sperm (12%). Due to lack of previous success with preserving electro-ejaculated semen in media free of animal-origin products, motility assays were performed to evaluate if spermatozoa retrieved from epididymides of plains bison can be cryopreserved in Andromed. Interestingly, cyropreserved epididymal spermatozoa had a higher motility than cryopreserved electro-ejaculated sperm after freezing-thawing procedures using a medium containing no products of source animal (respectively, 30% *v*. 7%). This result suggests that there may be a factor secreted by the reproductive accessory glands that interferes with the post-thaw survivability of bison sperm. In conclusion, this study supports the hypothesis that semen from plains bison behaves similarly to that of wood bison semen during cryopreservation and therefore could be used to establish protocols for advanced reproductive technologies in wood bison.

This project was supported by Canadian Adaptation and Rural Development in Saskatchewan.

225 DOES THE SUPPLEMENTATION OF THE BOAR SEMEN EXTENDER WITH CARNOSINE, L-HISTIDINE, AND TAURINE PRESERVE SPERM FUNCTION DURING STORAGE?

C. Matás[A], *J. C. Gardón*[B], *F. A. Garcia-Vazquez*[A], *S. Pacchini*[C], *and M. Ducci*[C]

[A]Department of Physiology, Faculty of Veterinary Science, University of Murcia, Murcia, Spain;
[B]Institute of Biotechnology Research, University of San Martin, Buenos Aires, Argentina;
[C]Department of Anatomy, Biochemistry and Physiology, Faculty of Veterinary Medicine, University of Pisa, Pisa, Italy

High levels of reactive oxygen species (ROS: superoxide, hydroxyl, hydrogen peroxide, nitric oxide, peroxynitrile) endanger sperm motility, viability, and function by interaction with membrane lipids, proteins, and nuclear and mitochondrial DNA (Sikka 2004 J. Androl. **25**, 5–18). ROS generation has a significant negative effect on the fertilization rate after IVF, and so measurement of ROS levels in semen specimens before IVF may be useful in predicting the IVF outcome (Agarwal *et al.* 2005 Fertil. Steril. **84**, 228–231). Several compounds of the antioxidant systems have been identified in the epididymal environment, spermatozoa, and seminal plasma. The antioxidants carnosine, L-histidine (Ducci *et al.* 2006 Pol. J. Vet. Sci. **9**, 159–163), and taurine (Van der Horst and Grooten 1966 Biochim. Biophys. Acta. **117**, 495–497) have been detected in boar semen and added to the extender in freezing procedures in several species. The main objective of this study was to evaluate the effect of carnosine, L-histidine, and taurine supplementation of the extender on boar sperm functionality as measured by sperm motility during computer-assisted semen analysis (CASA) and by IVF ability using mature oocytes, as previously described (Selles *et al.* 2003 Reprod. Domest. Anim. **38**, 66–72). The sperm-rich fraction from mature fertile boars was diluted with isothermal Beltsville thawing solution (BTS) extender. Diluted semen was placed at 15°C and centrifuged at 800*g* for 10 min. The semen pellet was resuspended with BTS supplemented by 5 mM of carnosine, L-histidine, or taurine or not supplemented (control) to provide 75×10^6 spermatozoa mL^{-1} and stored at 15°C for 24 h (IVF assay), or 48 or 120 h (for CASA assay). We observed that the motility parameters were affected by storage time and that the addition of taurine increased the motility at 48 h of storage. Alternately, the addition of L-histidine to the extender reduced significantly the motility parameters after 120 h. The results showed that the addition of L-histidine induced a significant ($P \leq 0.01$) decrease of the penetration rate (L-histidine 75.8% *v*. control 89.9%) and the number of sperm per oocyte penetrated (L-histidine 3.1 *v*. control 4.1). The rate of male pronuclear formation was not affected by the addition of antioxidants to the extender (over 85% in all cases). The addition of carnosine and taurine had no effect on the IVF parameters. In conclusion the antioxidants carnosine, taurine, and L-histidine affect sperm functionality differently, and further studies are necessary to elucidate what changes in sperm function take place during storage and the mechanisms by which these antioxidants exert their effects.

This work was supported by Italian-Spanish research project HI2005-0165 and AGL2006-03495.

226 DAY LENGTH DOES NOT AFFECT LIVE SPERM NUCLEAR SHAPE IN THE STALLION

J. J. Parrish[A], *C. Mueller*[B], *E. Ludwig*[A], *and J. L. Susko-Parrish*[A]

[A]University of Wisconsin-Madison, Madison, WI, USA;
[B]University of Wisconsin-Riverfalls, Riverfalls, WI, USA

Fourier harmonic analysis (FHA) of sperm nuclei is a precise and objective method to evaluate shape of the sperm head, with the calculated harmonic amplitudes highly related to male fertility. The FHA approach has been developed for use in the bull and the boar but has not yet been applied to the stallion. Direct utilization of the previous fluorescent approaches to identify and image live sperm nuclei in the bull cannot be used in the stallion due to the increased thickness of the post-nuclear region and thin anterior region of the sperm head. An alternative approach was developed in which live and motile sperm were isolated after filtration of an ejaculate through a Sephadex G-15 column. The resulting live sperm were sonicated briefly to separate tails and heads. The heads were isolated on a 45–90 discontinous Percoll gradient, fixed with paraformaldehyde (0.2%), centrifuged onto glass slides, and dried. The slides were then stained with eosin (1%), cleared with water, and dried again; Permount was added,