The Effect of Oxidative Stress on Equine Sperm Function, Semen Storage & Stallion Fertility

By Barry Ball, D.V.M.

Stallion infertility is an important problem in the equine industry. In the last 10 to 12 years, the application of transported semen and frozen semen in the horse has increased dramatically. However, of the stallions evaluated as candidates for frozen semen or chilled transported semen, approximately 25 to 40 percent are deemed unacceptable due to poor sperm survival after storage. There is little information regarding the reasons for the large variability in the quality of either frozen-thawed or chilled semen from different stallions. Currently, UC Davis researches are examining the importance of oxidative stress on stallion infertility and storage of either frozen or chilled semen.

Although all cells require oxygen for normal metabolism, this metabolic process also generates a number of oxygen metabolites that may be toxic to cells (oxidative stress). Oxidative stress is a well characterized mechanism associated with disease processes in many body systems. Research in other species indicates that production of these toxic oxygen metabolites (reactive oxygen species or free radicals) can be detrimental to sperm survival and fertility. Until now, there has been no research available to characterize the impact of oxidative stress on sperm function and fertility in horses. Current research by Dr. Barry Ball and Ph.D. candidate Ms. Julie Baumber includes 1) examining the ability of equine semen to produce reactive oxygen species, 2) investigating the antioxidant systems present in semen to counteract these oxygen by-products, and 3) determining the effect of reactive oxygen species on sperm function.

It appears that damage to equine sperm dramatically increases the production of reactive oxygen species. Therefore, any damage that might occur to sperm during freezing or transporting chilled semen would be expected to increase the production of reactive oxygen species which could, in turn, cause further detrimental effects on the remaining live sperm. In addition, abnormal sperm also produce a higher level of reactive oxygen species than normal sperm, which may be a cause of reduced fertility in stallions with a high percentage of abnormal sperm in their semen. Dr. Ball's laboratory has also determined that normal equine sperm possess a specialized mechanism to generate reactive oxygen species which may, in fact, play an important role in fertilization when this process is triggered at the appropriate time and in the appropriate amount.

For the second phase of their research, Dr. Ball and Ms. Baumber have characterized some of the enzyme systems that are present in semen specifically to degrade reactive oxygen species before they damage live sperm. One of these enzymes, catalase, is present in surprisingly high amounts in equine semen and its activity varies greatly between different stallions. Preliminary information by comparing catalase activity from stallions with high or low sperm motility suggests an association between this enzyme and sperm motility. Further studies will be required to confirm this observation.

The high activity of catalase in normal stallion's semen suggests that the reactive oxygen species that are produced are rapidly degraded before they have an adverse effect on sperm. In processing semen for either freezing or transportation as chilled semen, much of the enzyme is removed when sperm are separated from the liquid portion of semen (seminal plasma). This separation for semen processing may adversely affect the storage of sperm, particularly in stallions that have an increased production of reactive oxygen species in their semen. Future research will be directed to determine whether or not this mechanism accounts for the poor storage of sperm in some stallions.

Additional research in Dr. Ball's laboratory has addressed the effects of reactive oxygen species on equine sperm as well as the relative importance of different enzyme scavengers on protecting sperm from these free radicals. A number of reactive oxygen species are produced by different metabolic

processes or cell types. Ms. Baumber's research indicates that hydrogen peroxide is the most damaging reactive oxygen species relative to its effects on sperm function. Correspondingly, catalase, the enzyme that degrades hydrogen peroxide, protects sperm from hydrogen peroxide's damage and is present normally in high quantities in equine semen. Damage to equine sperm produced by reactive oxygen species appears first as a loss of sperm motility that occurs very rapidly after exposure to reactive oxygen species. The sperm's loss of motility appears to occur due to a disruption of the sperm's energy metabolism prior to death of the cells. This finding suggests that it may be possible to reverse the loss of motility associated with damage to sperm by reactive oxygen species.

Current studies are evaluating the effect of white blood cells on equine sperm. White blood cells normally produce reactive oxygen species in very high quantities, and the presence of even low numbers of white blood cells in equine semen may have a detrimental effect on semen quality. Researches will determine the number of white blood cells that must be present in semen to produce a detrimental effect on sperm as well as investigate the mechanism of damage to sperm by white blood cells.

Although much of the work conducted to date has a more basic nature, these experiments will form the cornerstone of understanding aspects of oxidative stress related to the production, degradation and effects of reactive oxygen species in equine semen. Future studies will examine the relationship of these parameters to both stallion infertility as well as help design new semen extenders and preservation methods for equine artificial insemination.