ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
CRYOPRESERVATION OF CANINE SEMEN-NEW CHALLENGES

Wenche Farstad
Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, P.O.Box 8146 Dep. N-0033 Oslo, Norway. E-mail: wenche.farstad@veths.no

Research in freezing technology of animal semen escalated with the discovery of the positive effect of the permeating cryoprotectant glycerol on spermatozoa during freezing and the beneficial effect of adding egg yolk to semen diluents for cooling to low temperatures. Glycerol has been widely used to preserve the fertility of sperm after freezing and thawing. The sperm plasma membrane serves as the main physical barrier to the outside environment. Plasma membrane destabilization during the freezing and thawing processes causes intracellular ice formation, which deforms the cell without breaking the plasma membrane. Glycerol may induce changes in the lipid packing structure of the sperm membrane and alter the stability and water permeability of the cell (Watson 1995).

Egg yolk (EY) protects cell membranes against cold shock and prevents or restores the loss of phospholipids from the membrane. EY has been widely used in semen extenders. It was added to TRIS-Glucose buffer (EYTG) and used for canine semen dilution and cooling up to 27 days with exchange of diluter (Verstegen et al. 2005). The addition of detergents, such as sodium dodecyl sulphate (SDS), seems to have a beneficial effect of in vitro longevity after thawing. Its effect on membrane fluidity seems mainly to be in interaction with EY but not through modification of membrane fluidity (Al Haider 2007). EY is not a defined entity, but a complex biological compound containing lipids, phospholipids, proteins, glucose, vitamins and antioxidants, which are all potentially useful for cell membranes (for review see, Huopalathi R et al. 2007) Unfortunately, EY is also a biologically hazardous compound in light of the recent spread of zoonotic diseases from birds, e.g. avian influenza. Hence, other chemically defined substances should replace EY for semen processing also in dogs.

Individual- and species differences exist in the ability of semen to tolerate freezing, which may explain part of the cryogenic success or failure (Farstad and Waterhouse 2006). The presence of long chained polyunsaturated fatty acids contributes to increased membrane fluidity. It was suggested that this relationship may be biphasic, i.e. that either too much membrane fluidity or too little may be damage to successful cryopreservation (Miller et al. 2005). Increased fluidity of the outer leaflet of the plasma membrane similar to the initial changes of sperm capacitation, has been detected in frozen-thawed dog spermatozoa by the use of flow cytometry (Al Haider 2007).

The protective effect of lipids may lie in the close association with the membrane rather than in modification or rearrangement of the membrane. This also points at lipids as an important, if not entirely new group of substances, which may substitute standard egg-yolk based diluents in preserving sperm survival during freezing (Farstad and Waterhouse 2006). Among these, lecithin, a fatty substance with emulsifying properties and composed of phosphoric acid, choline, fatty acids, glycerol, glycolipids and phospholipids, is an interesting candidate. Vegetable lecithin is currently investigated in order to avoid using substances of animal origin.

EY also contains antioxidants, which prevent cells and cell membranes from oxidative damage due to the generation of reactive oxygen species (ROS) (Bilodeau et al. 2000; Klinc and Rath 2007). An increasing number of publications now recognise the significance of protecting sperm from this damage during processing and freezing. Vitamins C and E (ascorbate and tocopherol), selenium, amino acids, BSA and a number of enzymatic antioxidant substances has been tested for freezing of human semen and for semen from several animal species from turkeys to buffalos.
Flow cytometry permits both the evaluation of several attributes simultaneously and the assessment of large numbers of spermatozoa (Graham and Mocé 2005, Al Haider 2007). One challenge lies in the ability to adapt standard freezing procedures to individuals, breeds and species based on the evaluation (e.g. by flow cytometry) of fresh semen. Secondly, the use of antioxidants in freezing diluents are being tested, but so far no standard recipe has been found that would uniformly protect sperm of all species or under all processing conditions. Thirdly, for sanitary reasons the substitution of EY in semen extenders with bioactive lipids or lipid containing compounds from synthetic- or plant derived sources, warrants further investigation.

References