4 DISCUSSION

After decades of hunting and unscrupulous interfering in natural habitats man has finally realized the importance of preserving natural habitats and the diversity of species. Assisted reproductive techniques can be a useful tool in restoring the numbers of wild animals that have been threatened to extinction by humankind around the globe.

The collection and preservation of genetic material of wild animals can ensure the protection of a wide genetic diversity within a population even when numbers might be small at times after unforeseen incidences. There were such events in the past such as the outbreak of Rinderpest, which reduced most populations of cloven-hoofed animals in South Africa to small numbers (De Vos 1987).

Nowadays the numbers of African buffaloes are very favourable again, but the possibility of reintroducing them to former habitats is limited. This is due to the introduction of exotic diseases, which are now endemic in certain areas of South Africa such as the Kruger National Park. The possible transmission of foot-and-mouth disease, corridor disease, tuberculosis and brucellosis limits the transport of buffaloes and therefore also minimizes the possibility of increasing the genetic variety outside these endemic areas. Assisted reproductive techniques such as artificial insemination (AI) and in-vitro fertilization (IVF) might enable the use of genetic material of "diseased" buffalo populations without transmitting the actual diseases.

In this study we compared two different semen extenders according to their abilities to preserve epididymal sperm during freezing and thawing, in respect of resulting motilities and acrosomal integrities. Triladyl™ is a widely used medium, which has been shown to be suitable for the freezing of epididymal African buffalo sperm. The reasons for comparing this to a new medium are the possible risks of disease transmission and variable hygienic standards that are inevitable when egg yolk containing semen extenders are used. AndroMed® is a totally defined medium free of animal products and has already been used to freeze epididymal sperm from the African buffalo (Herold *et al.* 2003a).

When semen extenders that are free of animal products were compared to conventional ones in the past no differences have been found in the post thaw-total motilites (Bousseau *et al.* 1998; Müller-Schlösser *et al.* 2001 and Müller-Schlösser *et al.* 1995). These studies were however conducted on ejaculated bull semen and must therefore cautiously be compared to our results. In our study most differences became only obvious during evaluation of the longevity, which has not been recorded in the previously mentioned trials.

Results of a previous study performed in our laboratory indicate that sperm frozen with Trilady™ has a better longevity than when AndroMed® is used (Herold *et al.* 2003b). The results of this trial can only confirm these findings. We observed hardly any significant differences between the two media immediately after thawing and only a few after one hour of storage after thawing. Two hours after thawing samples treated with Triladyl™ showed significantly higher motilities for 6 out of 8 different equilibration times used.

Hinsch *et al.* (1997) found no differences total and linear motility and non-return rates, when Triladyl™ was compared to Biociphos®. There were no significant differences in the total and linear motility over the 8 hour period of the trial. At 5 and 8 hours after thawing, the linear motility tended to be higher for semen frozen with Biociphos® (54 and 53%) than for semen frozen with Triladyl™ (28 and 26%) ($p<0.6$ and 0.7). In our present study also most of the differences became only obvious 2 hr after thawing. Whether an increased longevity has an influence on the non-return rate needs to be tested. During routine inseminations many cows are not inseminated at the optimal time and it would therefore not be surprising if longevity has a significant effect on non-return rates in field trials. We also compared the motility values before freezing, immediately, one and two hours after thawing amongst each other. Only the use of Triladyl™ resulted in post-thaw motilities as high as fresh ones. The total as well as the progressive motility was on the other hand lower even right after thawing, than it had been before freezing, when AndroMed® was used. Although not significant an obvious decrease over time in progressive motility after thawing could only be seen when AndroMed® was used. The values immediately and two hours after thawing were 14 ± 13 and 6 ± 9 respectively. Whereas the

progressive motilities immediately and two hours after thawing for the use of Triladyl™ were 17 ± 11 and 17 ± 11 respectively.

Results of previous studies did not show differences in acrosomal integritiy when Triladyl™ was compared to the egg yolk-free medium Biociphos® (Hinsch *et al.* 1997). We found significant differences for lost acrosomes, but considered these differences to be neglect able for two reasons. Firstly because of the low numbers recorded (between 0 and 12) and secondly because of the fact that neither intact nor damaged acrosome values differed significantly for the comparison of the same treatments.

We did neither find differences among equilibration times for intact or damaged acrosomes nor for the comparison of the media for the same equilibration times each. We found nevertheless Triladyl™ to result in superior numbers of intact acrosomes when equilibration times were ignored.

Since a high motility rate is one of the prerequisites for successful artificial insemination it must be concluded that Triladyl™ is superior to AndroMed®. Even though the risks of egg yolk-containing diluents are known, they have, to the knowledge of the author, never resulted in spreading of disease when Specific Pathogen Free eggs were used. As I believe the advantages of higher motilities to be bigger than the hygiene risks I must conclude with the suggestion to use Triladyl™ rather than AndroMed®.

Another aim of this study was to establish the influence of exposure time of sperm to semen extender before freezing. Furthermore I wanted to establish the ideal equilibration time for both media, if any difference was to be found.

When ejaculated bull semen was used it has been suggested in previous studies that sperm survival is the higher, the shorter the exposure time to glycerol is (Berndtson and Foote 1969 and O´Dell and Hurst 1956). Contrary to these reports are results that indicate no differences in the percentage of intact acrosomes and motile spermatozoa in bovine semen samples when equilibrated for 0.5 or 2 hours (Wiggin and Almquist 1975a).

Our results are similar to the latter mentioned, even though it can't be said that they confirm these previous findings, as we used epididymal rather than ejaculated sperm and we used equilibration periods for up to 9 hours and buffalo sperm has been used in our study. There were nevertheless no differences for neither acrosomal integrity nor motility for equilibration times between 2 and 9 hours. This was the case for both media used.

In previous trials epididymal sperm from the African buffalo has always been cooled and stored within the whole epididymis. In our experiment we flushed the sperm first and only then cooled, froze and stored it. Results of these before mentioned trials nevertheless show that epididymal sperm can resist temperatures of around 4°C and motility can be restored thereafter. Friedmann *et al.* (2000) report that epididymal sperm from the African buffalo can maintain motility when stored for up to 24 hours at 4°C. Similar results of another study show that when motility was measured every 12 hours during storage a significant decline could only be seen after 36 h (Kilian *et al.* 2000). Another study did not only confirm that there were no differences to the initial motility after a storage time of 24 h, it has also been shown there that similar post-thaw motilities could be achieved after 24 h of exposure to 4°C when compared to sperm frozen immediately (Gerber *et al.* 2001). Contrary to these findings a significant decline in motility within the first 8 h has also been reported when epididymides were treated the same way (Bezuidenhout *et al.* 1995).

As stated above these results must only be compared very cautiously to ours since whole epididymides were cooled then and flushed sperm was frozen and stored in liquid nitrogenin our experiment now.

It seems nevertheless apparent that epididymal sperm from the African buffalo is capable to survive at least up to 8 h at temperatures around 4°C and their motility can be restored.

This is important to know since sperm collection in this species is always done in the field and precludes ideal laboratory conditions.

Because a high rate of progressive motile spermatozoa is one of the prerequisites for successful AI it must be concluded that Triladyl™ is superior to AndroMed®. As I believe the advantages of higher motility to be bigger than the hygiene risks I

conclude that epididymal buffalo sperm should rather be frozen with Triladyl™ than with AndroMed®. I can further conclude from the presented results that any equilibration time between 2 and 9 hr is suitable to freeze epididymal sperm from the African buffalo.