



Spectrum: The SBS CryoSystem Proof of concept

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The SBS CryoSystem consists of a range of freezing extenders, allowing the clinician to identify the best extender and complementary freezing protocol for each individual stallion. Spectrum extenders feature various cryoprotectants and specific egg yolk or milk content that combine to protect sperm cells from damage caused by freezing, and protect the fertilizing ability of the semen after thawing. Each Spectrum extender formulation requires a specific freezing protocol to display its best performance.

To date there is no single universal cryopreservation protocol that is optimum for semen from all stallions. Use of a single protocol (extender, cooling rate, etc.) has led to the belief that stallions can be grouped into “good” and “bad” freezers. The SBS CryoSystem is based on the belief that semen from a large percentage of the stallion population can be frozen successfully if an effort is made to customize cryopreservation protocols to identify optimum conditions for each individual stallion.

In 2014, Select Breeders Service (SBS) and Minitube International have signed a strategic collaboration agreement committed to the advancement of the science of equine reproduction and the responsible development of the frozen semen industry. Since Minitube has introduced the SBS CryoSystem, a number of freezes with different stallions were performed to proof the Spectrum concept. All of the semen processing, freezing and post-thaw analysis was performed by an independent University laboratory.

In the following, results of 5 of these freezes are described and shown (total and progressive motility post thaw). In all of the freezes the ejaculates of three stallions were examined (stallions have normal sperm cells and are approved for breeding). All ejaculates were split equally and allocated to the different Spectrum extenders and the control extender (commercial milk based freezing extender). In each of the freezes, stallions showed a preference for a specific extender, although the specific freezing curve was not applied as recommended for the Spectrum extenders*. Trials performed comparing another freezing extender attested the variability in extender preference between individual stallions earlier. These test results confirm that the right choice of extender influences the freezing results and prove the Spectrum concept right.



* Tests samples were frozen in liquid nitrogen vapour. Applied protocol:

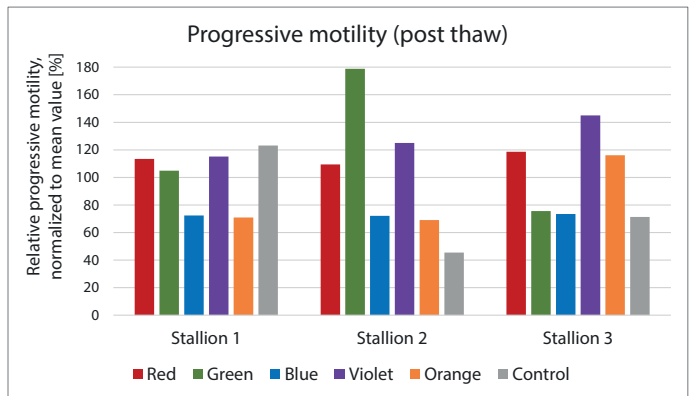
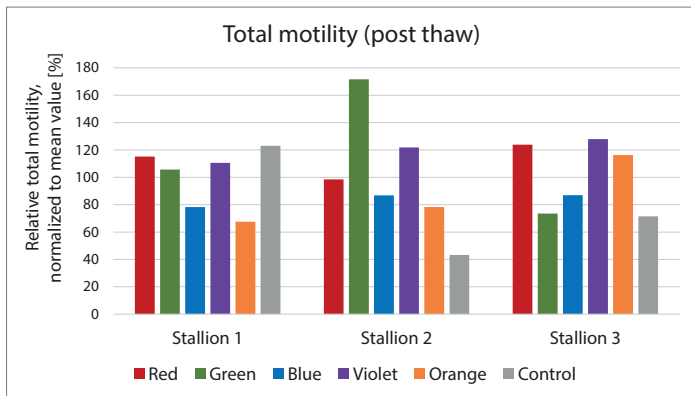
Cooling: Tube with semen in water cup (~ room temp.), 2h slow cooling in refrigerator (+5°C)

Straw filling: In cold handling cabinet at +5°C

Freezing: On straw rack 20 min over LN₂ vapour, then plunge in LN₂ storage container

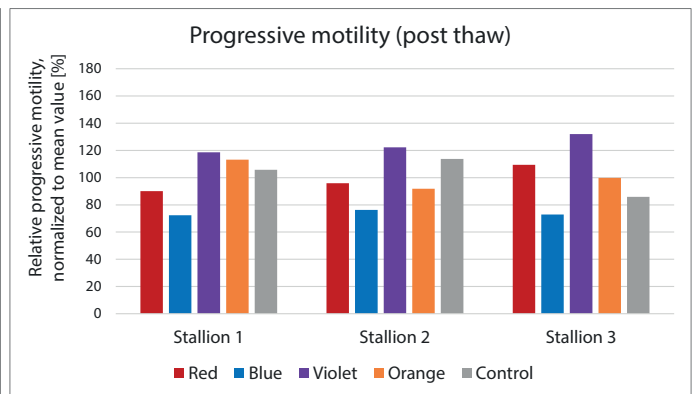
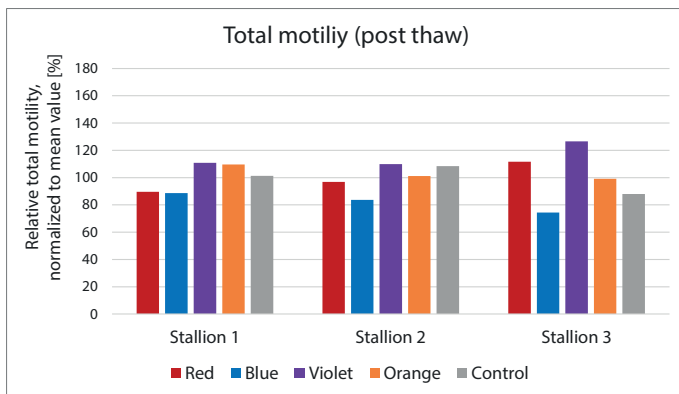
Test freeze 1

In the first freeze stallion 2 showed clear preference for Spectrum Green, while stallion 3 had slightly better progressive motility in Spectrum Red and Violet.



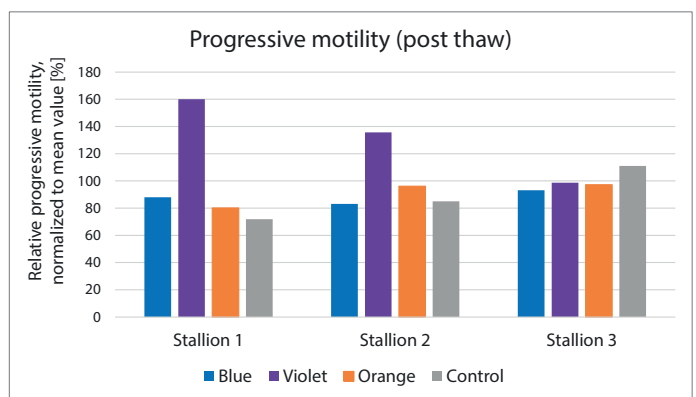
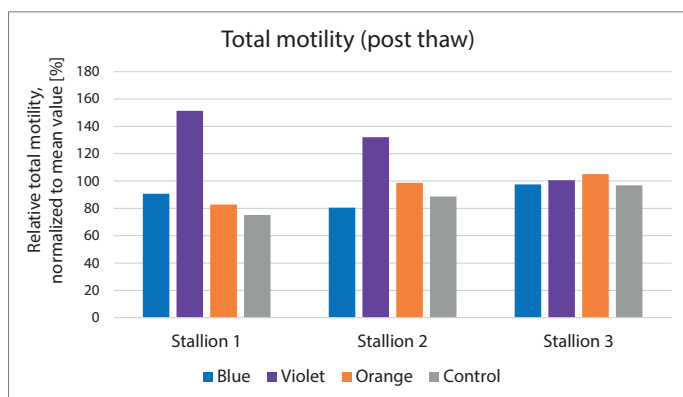
Test freeze 2

Test freeze 2 was performed with other three stallions. All of them showed a slight preference for Spectrum Violet.



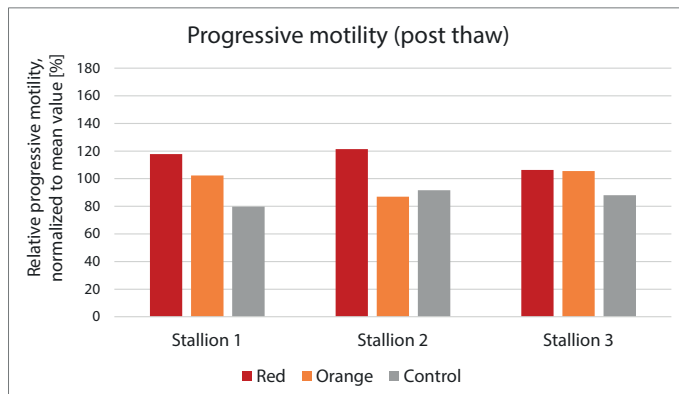
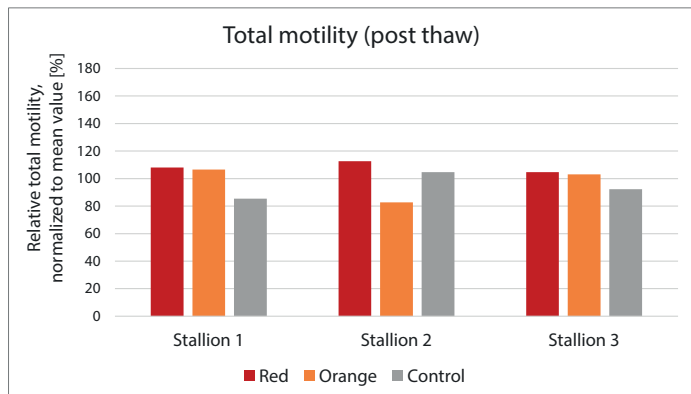
Test freeze 3

In another freeze test with 3 different stallions, one did not show a clear preference (3), while Spectrum Violet resulted in the best motility values for the other two stallions.



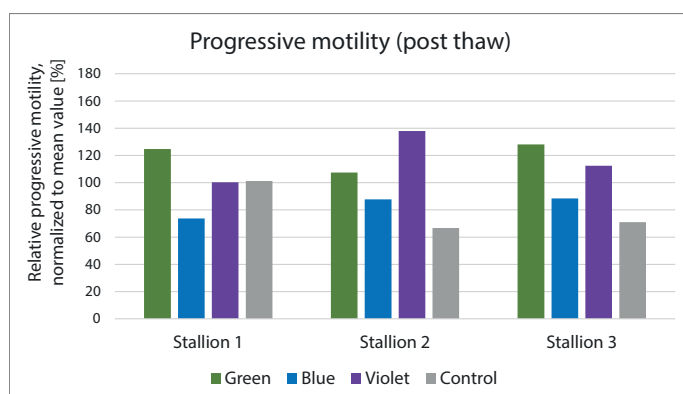
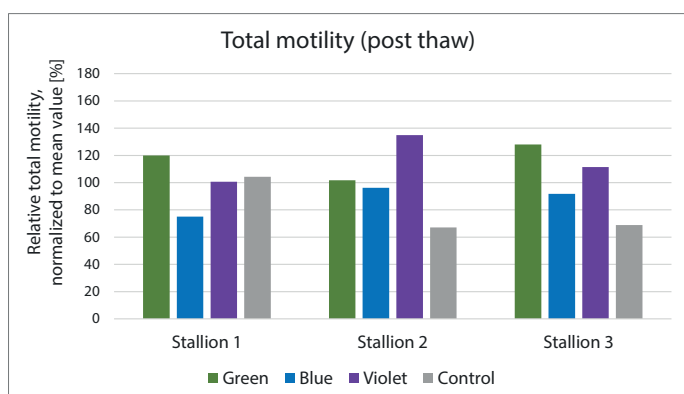
Test freeze 4

When the ejaculates of the same stallions of test freeze 3 were frozen in Spectrum Orange and Spectrum Red only, progressive motility was higher with Spectrum Red in two cases, while stallion 3 showed equal results in Spectrum Orange and Red. Spectrum Red combines best with a rapid cooling / freezing curve, and Spectrum Orange is recommended with a slow cooling / freezing curve. In our test freeze 4, both extenders were frozen with the same curve which might be the reason why both gave similar results. If the recommended freezing curve for each extender would have been applied, a preference for one of the two extenders might have been apparent. The control extender could not reach the motility values of any of the two Spectrum extenders.



Test freeze 5

When Spectrum Green, Blue and Violet were tested with the stallions of test freezes 3 and 4, a preference for Spectrum Green and Violet was respectively revealed.



Conclusions

The results of the freezes show that the use of a single extender for all stallions may lead to less than optimum results for many stallions. Within the breeding population exists high variability in the ability of sperm from individual stallions to survive cryopreservation. This is likely due to differences in the lipid composition of sperm membranes. With the Spectrum Test Freeze Kit and a split ejaculate trial, it is possible to find the best extender and protocol for each stallion. This results in more successfully frozen ejaculates, overall higher quality of frozen semen and more commercial doses per ejaculate.