Sperm survival following colloid centrifugation varies according to the part of the sperm-rich fraction used

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Spermatozoa present in the first 10 ml (portion 1, P1) of the sperm-rich fraction (SRF) of boar ejaculates show increased resilience to cooling and cryopreservation compared to those in the rest of the ejaculate (Saravia *et al.* 2008) presumably because of differences in exposure to seminal plasma. A new technique for selecting the most robust animal spermatozoa, Single Layer Centrifugation (SLC), has recently been developed at SLU (Morrell & Rodriguez-Martinez 2009). It was shown that SLC of SRF using Androcoll-P™ followed by storage at room temperature (22°C) in extender without antibiotics resulted in sperm preparations of high motility which survive longer than unselected sperm samples (Wallgren *et al.* 2008). However, it is not known how SLC using P1 instead of SRF would affect boar sperm survival. Furthermore, the technique requires scaling-up to enable larger volumes of ejaculate to be processed. Scaling up has been shown to be possible for stallion spermatozoa (Morrell *et al.* unpublished data).

In experiment 1, P1 from 12 ejaculates (4 boars, 3 ejaculates per boar) and 12 SRF from the same boars (again 3 ejaculates from each boar), collected on different occasions, were extended in Beltsville Thawing Solution (BTS) without antibiotics to achieve a sperm concentration of $100x10^6$ mL. Aliquots (1.5 ml) of these sperm samples were prepared by SLC on Androcoll-P™ by centrifugation at 300xg for 20 min. The resulting sperm pellets were washed in 5 ml BTS + bSA (5 mg/ml) by centrifugation at 500xg for 10 min. before resuspending in 1.5 ml of the same extender. The sperm suspensions were stored in a styrofoam box at room temperature (22°C) for up to 6 days. Sperm motility was assessed subjectively on a daily basis using phase contrast microscopy, after incubating the sperm suspensions at 38°C for 30 min. Differences between means were tested for statistical significance by analysis of variance, where $P < 0.05$ was considered significant.

In the second experiment ("scale-up"), larger volumes of extended semen were used, e.g. 3 ml or 4.5 ml were pipetted on top of 4 ml Androcoll-P™ and 10 ml, 12.5 ml or 15 ml extended semen were pipetted on top of 15 ml Androcoll-P Large, an optimized formulation for larger tubes. After centrifugation, the sperm pellet was resuspended in 3 ml or 10 ml BTS with bSA (1.25 mg/ml) respectively. Sperm motility was analysed by computer assisted sperm analysis (CASA) on 5μl aliquots of sperm samples placed in a pre-warmed Makler chamber (Sefi Medical Instruments, Haifa, Israel), depth 10 μm, using a Mika Cell Motion Analyzer (MTM Medical Technologies Montreux, Switzerland) and a microscope equipped with a warm stage and phase contrast optics (20x objective, Optiphot-2, Nikon, Japan). Two hundred spermatozoa per sample were examined.

In experiment 1, the mean differences in % sperm motility between control (non-SLC-selected) and SLC-selected samples were as follows: time 0 h, P1 +13.3%, SRF +16.6%; at 24 h, P1 +23.7%, SRF +23.4%; at 48 h, P1 +10%, SRF +24.6%; at 72 h, P1 +9.6%, SRF +39.2%; at 96 h, P1 +9.4%, SRF +39.2%; at 120 h, P1 +6.7%, SRF +25.8%. The non-SLC-

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selected samples showed bacterial growth after the first 24 h, which may have contributed to the subsequent deterioration in sperm motility. SLC-selected sperm samples from SRF had a higher motility than non-selected sperm samples and retained their motility for longer ($P < 0.001$), even at this relatively high storage temperature (22°C) without antibiotics. SLC-selected spermatozoa from SRF had significantly better motility than those from P1 at 24, 48, 72 and 96 h ($P < 0.01$), although not at 0 or 120 h ($P > 0.05$), and retained their motility longer. SLC-selected spermatozoa from P1 were significantly better than controls only at 0 and 24h ($P < 0.01$).

For experiment 2, there were no differences in yield or motility when 3 ml or 4.5 ml extended ejaculate was used on 4 mL Androcoll-P™ nor between 10 ml, 12.5 ml or 15 ml on 15 ml Androcoll-P™-"Large". In a further experiment where 4.5 ml were compared with 15 ml on the "small" and "large" colloids respectively, there was no difference between the "small" and "large" preparations (sperm motility: "small" = 81.7 ± 7.7, "large" = 84.9 ± 6.1%; yield: "small" = 43.7 ± 10.1%, "large" = 42.4 ± 23%). The motility of the unselected spermatozoa in these experiments was 77.4 ± 19%. It was interesting to note that the yield of spermatozoa was lower than in the previous experiment (performed 15 months earlier) which may be due to deteriorating quality in the semen of boars as they age.

The findings indicate that SLC selected the most motile spermatozoa from both P1 and SRF. Moreover, SLC-selection removed bacterial contamination occurring during semen collection, enabling the sperm suspensions to be kept without antibiotics in the semen extender or requiring a reduced temperature. SLC-selection of SRF resulted in better sperm motility than SLC of P1 suggesting a potentially critical relationship between initial sperm numbers and accessory gland secretions. These observations are of interest to the swine AI-industry for the following reasons: (i) reducing the use of antibiotics in semen extenders; (ii) simplifying sperm storage requirements; (iii) providing additional information about the effect of seminal plasma on boar sperm survival. Further studies on all these issues are warranted. Finally, the results of the second experiment showed that it was possible to scale-up the volumes of semen used from the 1.5 ml used in the first experiment to 15 ml. This experiment is continuing, to scale-up to even larger volumes and to assess other parameters of sperm quality.

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References

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