Table 1
Mean values of total motility (TM), progressive motility (PM), rapid cells (RAP) and plasma membrane integrity (PMI) analyzed at 0 (0h), 24 (24h) and 48 hours (48h)

	0h	24h				48h			
		C	G1	G2	G3	C	G1	G2	G3
TM (%)	76 ± 9	50 ± 21	63 ± 16	62 ± 18	62 ± 16	33 ± 21[a]	49 ± 17[b]	49 ± 15[b]	50 ± 21[b]
PM (%)	37 ± 9	25 ± 17	34 ± 15	35 ± 16	34 ± 15	15 ± 13	24 ± 14	24 ± 14	25 ± 15
RAP (%)	68 ± 11	42 ± 22	54 ± 18	55 ± 19	54 ± 18	27 ± 19[a]	43 ± 17[b]	42 ± 19[b]	43 ± 20[b]
PMI (%)	56 ± 13	53 ± 14	60 ± 11	60 ± 9	59 ± 11	41 ± 13[a]	56 ± 9[b]	55 ± 11[b]	53 ± 12[b]

[a,b] Values in the same row with different superscript differ significantly ($P < 0.05$).

substrates. LC has a powerful antioxidant effect by reducing the availability of lipids for peroxidation and increasing antioxidant enzyme activity such as *superoxide dismutase* and *glutathione peroxidase*. Moreover, carnitines ensure the operation of oxidative pathways by reducing acetyl-CoA levels, and provide acetyl groups for sperm motility. Despite these findings, there are no reports about the use of LC and AC in cooling extenders for equine semen. The aim of this study was to evaluate the effect of these substances on cooled sperm viability. Two ejaculates of 10 stallions each were used. After collection, the ejaculates were evaluated for volume, motility and concentration, and then diluted with skim milk-based extender (Botu-Semen) to a final concentration of $50x10^6$ spermatozoa/mL. Ejaculates were divided into 4 groups: Control (no LC/AC), G1 (0,05mM/mL of LC+0,05mM/mL of AC), G2 (0,1mM/mL of LC+0,1mM/mL of AC) and G3 (0,15mM/mL of LC+0,15mM/mL of AC). Thereafter, the samples were kept at 5°C for 24 and 48 hours. Spermatozoal variables evaluated were: movement kinetics (Hamilton Thorne CASA), and plasma membrane integrity by epifluorescence microscopy with the fluorescent probes carboxyfluoresceine and propidium iodide. Evaluations were performed in fresh semen (0h), and at 24 hours (24h) and 48 hours (48h) of cooling. Statistical analyses were performed by ANOVA followed by Tukey test and the level of significance was set at $P < 0.05$. After 48 hours of storage treated groups yielded significantly higher percentages of TM, RAP and PMI than the control ($P < 0.05$) while at 0h and 24h no significant differences occurred. There was no statistical difference between treated groups at any time. In conclusion, the combination of L-carnitine and acetyl-L-carnitine enhanced the maintenance of sperm motility and provided a better protection of the plasma membrane after 48 hours of storage at 5°C.

Preliminary results using a new container for cooled stallion semen transport

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Since nearly all horse breed registries authorize the use of artificial insemination, shipped cooled semen is used on a large scale in many countries. Nowadays there are different types of containers to transport semen, ranging from sophisticated devices to more simple styrofoam boxes. This study aimed to verify the effectiveness of a thermal polyethylene bag and to compare it with other semen transport devices during storage for 8 hours. Six ejaculates from three fertility-proven stallions were used. The semen was diluted 1:1 with skim milk and split in 4 aliquots. The semen was analyzed at 0 h (control group) and at 8 h in 3 different chilling devices which were Equitainer (EG), Botuflex (BG) and thermal polyethylene bag (TBG). Sperm motility (%) and vigor (0-5) were evaluated with a light microscope by an experienced technician. Sperm concentration was measured using a SpermCue photometer. Membrane integrity was tested by CFDA/PI staining and the membrane functionality by HOST. Statistical analysis was performed by ANOVA and the comparison of means was performed using Least Significant Difference (LSD). Fresh semen (CG) had better motility (64%) and vigor (2.2) when compared to all cooled aliquots. However, there was no difference in the same parameters between the cooled-stored semen in each container (EG 39% and 1.6; BG 37% and 1.5; TBG 35% and 1.5). Functionality of the membrane did not differ between semen aliquots. Membrane integrity in TBG (69%) was the same as in the CG (61%) but TBG and CG were different ($P < 0.05$) from EG (73%) and BG (72%). Use of a thermal polyethylene bag for the transportation of stallion semen did not harm sperm quality when compared with other types of container. In addition, this bag seems to be a competitive alternative for cooled semen transport due to the low cost and the size of a large envelope. Since there was no damage in the evaluated characteristics of semen stored in the thermal polyethylene bag, this alternative container can be recommended for equine cooled semen transport for up to 8 hours.

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In Vitro culture of equine spermatogonial cells in a suspension cell system

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Spermatogenesis is one of the most complex and longest processes of sequential cell proliferation and differentiation