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P35 | Optimization of cryopreservation of Arabian stallion sperm using dimethylformamide, glycerol, and different freezing protocols

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This study evaluated the effect of penetrating cryoprotectant agents (CPA) and the cryosurvival of three freezing protocols on the kinematics and integrity of membranes of frozen-thawed stallion sperm. Twenty-four ejaculates of four adult Arabian horses were collected in six weekly sessions (six ejaculates/horse). Each ejaculate was divided into two aliquots. With the first aliquot, three CPA treatments were conformed: 5% glycerol (GLY), 5% dimethylformamide (DMF), and 3%–3% DMF–GLY combination, and the sperm samples were frozen exposing them to liquid nitrogen (LN₂) vapors. The second aliquot was diluted with freezing medium plus 5% DMF and the sperm samples were frozen in three freezing protocols: (P1) Styrofoam cryo-box (30×29×31 cm of length, width, and height, respectively) with two ramps (at 17 and 7 cm above LN₂); (P2) freezing unit® (*Minitüb, Germany*); and (P3) programmable TK 4000-freezer® (*Compacta, Brazil*). The DMF–GLY combination and DMF yielded higher ($p < .05$) post-thaw values than the GLY regarding the motility (SM: 54.2 ± 2.25 and 50.2 ± 1.80 vs. $41.4 \pm 2.35\%$, respectively), curvilinear velocity (VCL: 58.0 ± 1.71 and 54.0 ± 1.58 vs. $42.3 \pm 1.60 \mu\text{m/s}$), and the proportion of sperm with intact plasma and intact acrosome (IPIA: 58.0 ± 1.11 and 52.6 ± 0.99 vs. $42.5 \pm 1.07\%$). Furthermore, the P1 protocol produced a similar ($p > 0.05$) post-thaw SM, VCL, and IPIA than the other protocols. Indeed, the P1 and P3 protocols yielded lower proportion ($p < 0.05$) of sperm with damaged plasma and damaged acrosome than the P2 protocol after thawing (3.7 ± 0.18 and 3.1 ± 0.18 vs. $6.1 \pm 0.44\%$, respectively). In conclusion, the addition of DMF or combined with GLY to freezing medium, and the freezing with Styrofoam cryo-box with two ramps increase the cryosurvival of Arabian stallion spermatozoa.

P36 | Potential benefits of maternal Hydroxytyrosol supplementation during late pregnancy on calf growth

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Cow nutrition during late gestation could play a key role on fetal development. Additionally, the antioxidant properties of hydroxytyrosol could act as a therapeutic agent to control cow metabolic stress during pregnancy. Fifty-seven Parda de Montaña and 46 Pirenaica pregnant dams (PA vs PI) were blocked into 2 groups according to the nutritional treatment applied during the last third of gestation. One group fed 100% of the nutritional requirements and the nutrient-restricted group received 60% (100% vs 60%). Furthermore, the diets of half of the animals in each group were supplemented with hydroxytyrosol (HT vs Control). After calving, all dams fed a 100% diet. Calves were fed on maternal milk and dam milk yield was recorded at day 23 postpartum. Data were analyzed with generalized linear models with nutritional treatment, hydroxytyrosol supplementation, breed and gender (in calves) as fixed effects. Nutritional treatment in late gestation influenced dam weight (660 vs 613 kg for 100% and 60% dams, $p < 0.001$) and body condition score (3.4 vs 2.9 for 100% and 60% dams, $p < 0.001$) at calving, as well as milk yield in PA dams (11.9 vs 10.1 kg for 100%-PA and 60%-PA, $p = 0.014$) with no effects of HT supplementation on dam parameters. In contrast, calves from HT-supplemented dams were heavier at birth (49.1 vs 46.2 kg for HT and Control calves, $p = 0.003$) and these differences remained at one month of age in 100%-PI calves (89.5 vs 77.6 kg for 100%-HT-PI and 100%-Control-PI calves, $p = 0.029$). Despite HT supplementation had no evident effects on dam performance, its inclusion in the dam diet stimulated fetal growth with postnatal effects during lactation. Funded by PID2020-113617RR-C21.