water bath (38°C/20-s), pooled and assessed after 30 and 150 min of incubation at 38°C. Motility was recorded and analysed in a CASA system and sperm viability, acrosomal status, mitochondrial activity, apoptosis and free radicals (ROS) were assessed by flow cytometry after staining with propidium iodide, PNA-FITC (peanut agglutinin), Mitotracker deep red, Annexin V and CM-H2DCFDA respectively, using Hoechst 33342 to discard debris. Data were analysed with the R statistical environment v.3.4.3. The effects of age and incubation on sperm post-thawing quality were determined by using linear mixed-effects models. The boar age did not influence any of the parameters under study ($p > 0.05$). Incubation of the samples at 38°C for 150 min caused a general decrease in sperm quality, affecting total motility ($p < 0.001$), progressive motility ($p < 0.001$), viability ($p < 0.001$), number of viable sperm with damaged acrosome ($p < 0.001$), mitochondrial membrane potential ($p < 0.001$), apoptosis ($p < 0.003$) and ROS ($p < 0.022$). In conclusion, our findings demonstrated that post-thawing quality of Gochu Asturcelta sperm was affected by incubation time after thawing. Supported by: INIA RZP2013-00006-00-00 and SENECA foundation 19892/GERM/15.

P20 | Effectiveness of two-step accelerating cooling rate on post-thaw characteristics of ram sperm

DA Galarza[1,2]; A López-Sebastián[2]; J Santiago-Moreno[2]

[1]*Facultad de Ciencias Agropecuarias, Universidad de Cuenca, Cuenca, Ecuador;* [2]*Departamento de Reproducción Animal, INIA, Madrid, Spain*

A ram sperm cryopreservation protocol was optimised via assays involving different cooling rates through a programmable biological freezer. Ninety-eight ejaculates semen from twelve adults Merino rams were cryopreserved by using Protocol 1 (three-step decelerating cooling rate) from 5 to −35°C (40°C/min), from −35 to −65°C (17°C/min), and then from −65 to −85°C (3°C/min); Protocol 2 (three-step accelerating cooling rate), from 5 to −5°C (4°C/min), from −5 to −110°C (25°C/min), and then from −110 to −140°C (35°C/min); or Protocol 3 (two-step accelerating cooling rate), from 5 to −10°C (5°C/min), and then from −10 to −130°C (60°C/min). The results showed that Protocol 3 yielded greater post-thawed sperm motility values than Protocol 2 ($p < 0.05$) and Protocol 1 ($p < 0.01$): 61.4 ± 1.9%, 47.9 ± 2.0% and 44.5 ± 0.9%, respectively for total sperm motility; 27.2 ± 1.0%, 20.8 ± 1.1% and 18 ± 1.0%, respectively for progressive motility. Similarly, the percentage of sperm with intact plasma, acrosome and mitochondrial membranes [PI(-)/PNA-FITC(-)/MITO(+)] was higher ($p < 0.05$) in sperm that underwent the Protocol 3 (58.4 ± 1.1%) than Protocol 2 (45.8 ± 1.5%) and Protocol 1(41.7 ± 1.3%). Moreover, Protocol 3 yielded lower ($p < 0.05$) DNA damage than Protocol 1 (2.3 ± 0.5% vs 7.1 ± 1.2%). In conclusion, the two-steps accelerating cooling protocol provides advantages over the three-steps freezing protocols for cryopreservation of ram sperm. This research was support by IMAGE European project N°677353.

P21 | Long storage time in liquid nitrogen negatively affects post-thawing sperm quality in dogs

C Ródenas[1]; E Molina[1]; I Parrilla[2]; X Lucas[2]

[1]*Veterinary Hospital Teaching, University of Murcia, Espinardo, Murcia, Spain;* [2]*Department of Animal Medicine and Surgery, University of Murcia, Espinardo, Murcia, Spain*

Frozen semen is an important tool for canine breeding programs because it allows the storage of semen doses of valuable stud dog for an unlimited period of time in liquid nitrogen (LN). However, loss of sperm quality in semen doses long time stored in LN has been documented in human, porcine and bovine. Thus, this study aimed to evaluate whether a long storage time in LN also affects the quality of frozen-thawed canine spermatozoa cryopreserved using the Uppsala method. With compliance owner´s dogs, frozen semen doses of 8 males of different breeds stored in canine semen bank of Veterinary Teaching Hospital were grouped according to the storage time in LN: G0 (17 straws, 3–4 weeks of LN storage; Control Group), G1 (12 straws, 8–11 years) and G2 (5 straws, 12–17 years). Straws were thawed at 38°C/20 s and sperm quality was evaluated at 10 min post-thawing. Total (TM) and progressive motility (PM) were assessed using a CASA system. Sperm viability (V; sperm with intact plasma and acrosome membranes) was assessed using flow cytometry after labeling sperm with H-42, PI and FICT-PNA. The post-thaw sperm quality declined as storage time in LN increased: TM (74.0 ± 2.07, 68.92 ± 2.63 and 59.60 ± 9.17 for G0, G1 and G2, respectively; $p < 0.1$), PM (57.25 ± 2.98, 46.17 ± 3.41 and 29.40 ± 8.97 for G0, G1 and G2, respectively; $p < 0.05$) and V (71.18 ± 3.97, 61.32 ± 3.78 and 54.67 ± 1.0 for G0, G1 and G2, respectively; $p < 0.1$). In conclusion, post-thaw canine sperm quality is affected by long storage time in LN, being PM the sperm parameter most affected. Supported by Seneca Foundation (19892/GERM/15).

P22 | Effect of seminal plasma on kinetic parameters of donkey spermatozoa incubated with exogenous hydrogen peroxide

M Papas[1]; N Puigdollers[1]; J Catalan[1]; S Bonilla[1]; S Gacem[1]; J Miró[1]; M Yeste[2]

[1]*Equine Reproduction Service, Department of Animal Medicine and Surgery, Faculty of Veterinary Sciences, Universitat Autònoma Barcelona, Spain;* [2]*Department of Biology, Faculty of Sciences, University of Girona, Girona, Spain*

This study aimed at evaluating the response of donkey spermatozoa to oxidative stress induced by exogenous hydrogen peroxide (H_2O_2). We also determined whether the presence of seminal plasma (SP) is able to modulate the sperm response to that stress. With this purpose, a total of nine ejaculates were collected from three jackasses and extended in a skimmed milk-based extender (Kenney). Each ejaculate was split into two aliquots. In the first one, SP was not