

COC) compared to Ex (0.02 ± 0.007 and 0.25 ± 0.05 nmol/COC, respectively; $p < 0.05$). Surprisingly, Cp COCs consumed high amounts of glutamine (132.5 ± 128.5 nmol/COC), while Ex COCs produced it (-1.2 ± 0.6 nmol/COC; $p < 0.05$). Our results show that Ex and Cp COCs differently use the fatty acids and amino acids present in the maturation medium and could be related to their divergent meiotic competence. Further studies are needed to fully unveil these mechanisms.

P 77 | Epididymal storage and N-acetylcysteine supplementation on chromatin status of cryopreserved stallion epididymal spermatozoa

S González Hidalgo¹; E Matilla²; B Macías-García²; FM Sánchez-Margallo²; F Martínez-Pastor¹

¹Institute of Animal Health and Pastoral Farming Development (INDEGSAL), University of León, León, Spain; ²Assisted Reproduction Unit, Minimally Invasive Surgery Centre Jesús Usón (CCMIJU), Cáceres, Spain

Epididymal spermatozoa is a post-mortem source of germplasm from valuable stallions. However, recovering can take place in the field, requiring the refrigerated storage of epididymes before sperm cryopreservation. Moreover, sperm DNA fragmentation can occur during the freezing/thawing process, being related to oxidative stress. Thus, we assessed the effect of refrigerated storage of epididymes and antioxidant supplementation in the post-thawing chromatin status of stallion spermatozoa. Epididymides were retrieved from 4 horses castrated for veterinary reasons and preserved for 24 or 48 h at 4°C; then, spermatozoa were harvested and frozen in INRA 96 (2.5% egg yolk, 2.5% glycerol, 2.5% dimethylformamide), added with 0, 0.1, 1 and 2.5 mM of the antioxidant N-acetylcysteine (NAC). After thawing, chromatin status was assessed by Sperm Chromatin Structure Assay (SCSA, flow cytometry), obtaining DNA fragmentation (%DFI) and chromatin immaturity (%HDS). The effect of male, refrigeration time and NAC concentration were analyzed by GLM (results as mean \pm SEM). %DFI was low ($2.12\% \pm 0.28$) and affected by male and NAC ($1.67\% \pm 0.23$ with 2.5 mM, $p < 0.05$), not being affected by refrigeration time. %HDS was also low ($2.99\% \pm 0.38$), and it was affected by an interaction male \times time, but not by NAC. Overall, %HDS decreased from 24 h ($3.80\% \pm 0.40$) to 48 h ($2.10\% \pm 0.41$). In conclusion, equine epididymal spermatozoa could withstand refrigerated storage up to 48 h with minimal chromatin alterations. Chromatin status seems to be defined by the male, and NAC at 2.5 mM could prevent DNA damage in the freezing/thawing process. Therefore, NAC might be considered for supplementing freezing media for horse epididymal samples, especially from males with susceptibility to sperm DNA damage.

P 78 | Honey bee may substitute egg yolk in ram semen extenders

A Gracia¹; E Ferraz¹; N Martin¹; F Gonzalez¹; F Cabrera¹; A Medrano²

¹Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain; ²National Autonomous University of Mexico, Cuautitlán Izcalli, Mexico

The objective of this study was to test whether the substitution of egg yolk by honey bee in the extenders for ram semen may preserve sperm quality during refrigeration at 5°C. This work was conducted in Gran Canaria, Spain (28°N). Semen was collected from 4 Canary rams using artificial vagina. Sperm concentration was adjusted to 400×10^6 cells/ml by adding the correspondent extender. After dilution, progressive motility (visual), plasma membrane integrity (eosin/nigrosine), functionality (HOST), and acrosome integrity (phase-contrast), were assessed. Firstly, egg yolk (20%) was replaced by honey which was added at: 10, 5 or 2.5%. Secondly, the inclusion of honey at 2.5% (i) without, or (ii) with egg yolk (20%) was tested. Diluted semen was slowly cooled to 5°C, and kept at that temperature for 48 h; sperm quality was assessed 24 and 48 h after cooling. Sperm variables were analysed for differences within and between treatments. Stage I. Motility and plasma membrane-function of spermatozoa at 24 and 48 h were similar in the control, 2.5 and 5% honey; 10% honey showed lower values ($p < 0.05$). There were no differences in the other variables. Stage II. Motility of spermatozoa at 24 and 48 h were similar in the control and 2.5% honey plus egg yolk; 2.5% honey without egg yolk showed lower values ($p < 0.05$). There were no differences in the other variables. In conclusion, honey bee (2.5 and 5%) may substitute both egg yolk and glucose in ram semen extenders during refrigeration for 48 h.

P 79 | Effects of FSH and nutrition on the luteal angiopoietin (Angpt) system in ewes

A Gram¹; M Kowalewski¹; A Reyaz²; V Valkov²; D Redmer²; A Grazul-Bilska²

¹Institute of Veterinary Anatomy, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; ²Department of Animal Sciences, North Dakota State University, Fargo, USA

The CL is a transient endocrine gland and one of the fastest growing and highly vascularized tissues. Its development and function are affected by various factors, including hormones and plane of nutrition. Recently, the effects of arginine (Arg) and diet plan on luteal vascular development have been addressed. However, their effects on one of the important complementary systems, i.e., Angpts, have not yet been investigated. Therefore, the aim of this study was to evaluate the expression of Angpt-1, -2, and the Tie2 receptor in CL of (Exp. 1) ewes administered with Arg or saline, and (Exp. 2) in animals treated with FSH, and fed a control, excess or restricted diet in both experiments. Samples were obtained from early, mid- and/or late-luteal phase of the estrous cycle. Immunohistochemistry (IHC) and qPCR were used. Localization of Angpt1 and Tie2 was observed in capillaries and larger blood vessels, while Angpt2 was present in larger blood vessels. Tie2 was also localized in small and large luteal cells. Protein expression of Angpt1 was affected by diet and luteal phase; Tie2 protein was luteal stage dependent. Angpt-1 and -2, Tie2, and Angpt1/Angpt2-mRNA ratio, were all affected by the luteal phase, but not by diet or Arg (Exp. 1), and by phase and diet in FSH-treated ewes (Exp. 2). When compared with saline treatment, FSH affected Angpt-1 and/