Abstracts

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493. Effects of Hypotaurine, Cysteamine, and Aminoacids Solution on Post-Thaw Microscopic and Oxidative Stress Parameters of Angora Goat Semen. Kenan Coyan, Purhan B. Tuncer, Serpil Sariozkan, Pinar A. Ulutas, Mustafa Numan Bucak, Nuri Baspinar, and Birol Ozkalp. Selcuk University, Konya, Turkey; Ministry of Agriculture and Rural Affairs, Lalahan, Turkey; Adnan Menderes University, Aydin, Turkey

This study was conducted to determine the effects of cysteamine, hypotaurine and aminoacids solution (BME) on standard semen parameters, lipid peroxidation and antioxidant activities of Angora goat semen after the freeze-thawing process. Ejaculates collected from four Angora goats were evaluated and pooled at 37°C. Semen samples, which were diluted with a Tris-based extender containing the antioxidants hypotaurine (5 mM) and cysteamine (5 mM), and an aminoacid solution (2.5%), and an extender containing no antioxidants (control), were cooled to 5°C and frozen in 0.25 ml French straws in liquid nitrogen. Frozen straws were thawed individually at 37°C for 20 s in a water bath for evaluation. Supplementation with cysteamine, hypotaurine and BME caused significant ($P < 0.05$) increases in sperm motility, and significant ($P < 0.05$) decreases in total abnormality rates in comparison to the control group. While all in vitro treatments did not affect the acrosomal abnormality rates, hypotaurine and BME but not cysteamine significantly ($P < 0.05$) increased the HOST results as compared to the control group. Supplementation with antioxidants and BME did not significantly affect MDA levels and CAT activity in comparison to the control group ($P > 0.05$). The antioxidants hypotaurine and cysteamine increased SOD activity when compared to the BME and control groups ($P < 0.05$).

494. Acrosome Reaction Induced by Zona Pellucida Is Mediated by Alpha-7 Nicotinic Acetylcholine and Epidermal Growth Factor Receptors. Yael Hillman and Haim Breitbart. Bar-Ilan University, Ramat-Gan, Israel

In order to penetrate into the egg, the capacitated spermatozoon should bind to the zona pellucida (ZP) and undergo the acrosomal reaction. The acrosome reaction is mediated by receptor activation that leads to calcium influx into the sperm. In the present study we showed that isolated ZP activates sperm alpha7-nicotinic-achetylcholine receptor (α7nAChR) leading to the trans-activation of epidermal-growth-factor-receptor (EGFR), calcium influx and the acrosome reaction. The data revealed that alpha7nAChR agonist induces the acrosome reaction which was inhibited by EGFR-antagonist, suggesting that EGFR mediates this reaction. Moreover, the ZP induced acrosome reaction was significantly inhibited by alpha-bungarotoxin (alpha7 antagonist) and by

sperm exhibiting a characteristic pattern of capacitation was visualized by chlortetracycline staining and tyrosine phosphorylation in each experimental group. In our third experiment, we examined how sperm from each experimental group might affect developmental competence of an embryo produced by ICSI, including cleavage, development to the blastocyst stage, and chromosomal integrity of the blastocyst stage. ROS produced by mitochondria was decreased by cccp. Mitochondrial integrity, as assessed by mitotracker red was intact in all group and the proportion of active mitochondria as indicated by JC-1 was 82.4%, 83.3% and 33.3%, in the control group, Act group and MT group, respectively ($P<0.05$). The proportion of sperm exhibiting the characteristic pattern of capacitation, as determined by chlortetracycline staining, was 58.3%, 71.4% and 71.4% in the control group, Act group and MT group, respectively, whilst levels of tyrosine phosphorylation were 0.0%, 26.9% and 61.8% ($P<0.05$). There were no significant differences in the rate of embryo development to the blastocyst stage among the three groups (20.7% vs 25.0% vs 31.4% respectively, $P>0.05$). On the other hand, chromosomal integrity of the blastocyst stage in the MT and control groups were higher than in the Act group (78.6%:11/14 vs 86.7%:13/15 vs 7.1%:1/14, respectively, $P<0.05$). Our results indicate that suppression of mitochondorial activity in activated bovine sperm reduce ROS production but induce normal embryogenesis following ICSI.

496. The Effect of Antioxidants on Post-Thawed Angora Goat (*Capra hircus ancryrensis*) Sperm Parameters, Lipid Peroxidation, and Antioxidant Activities. Purhan Barbaros Tuncer, Mustafa Numan Bucak, Serpil Sariozkan, Fatih Sakin, Ahmet Atessahin, Recai Kulaksiz, and Mesut Cevik. Ministry of Agriculture and Rural Affairs, Lalahan, Ankara, Turkey; Erciyes University, Kayseri, Turkey; Afyon Kocatepe University, Afyon, Turkey; Firat University, Elazig, Turkey; Ankara University, Ankara, Turkey; Ondokuz Mayis University, Samsun, Turkey

The aim of this study was to determine the effects of the antioxidants curcumin, inositol and carnitine on microscopic seminal parameters, lipid peroxidation (LPO) and the antioxidant activities of sperm, following the freeze-thawing of Angora goat semen. Ejaculates were collected via artificial vagina from three Angora goats and microscopically evaluated and pooled at 37°C. The pooled semen samples were diluted in a Tris-based extender, including curcumin (2.5, 5 or 10 mM), inositol (2.5, 5 or 10 mM), carnitine (2.5, 5 or 10mM) and no antioxidant (control). The diluted semen was slowly (at a rate of 0.2-0.3°C/min) cooled to 5 °C and then cryopreserved in 0.25mL French straws. Frozen straws were thawed individually at 37°C for 20 s in a water bath, for microscopic sperm evaluation. The freezing extender supplemented with 2.5mM curcumin led to higher percentage of computerassisted semen analyzer (CASA) sperm motility (65±3%), when compared to the control, inositol and the

10mM carnitine ($P < 0.01$) groups, following the freeze-thawing process. The addition of antioxidants did not provide any significant effect on the percentages of post-thaw subjective analyses and CASA progressive motilities, as well as sperm motility characteristics (VAP, VSL, LIN and ALH), compared to the controls. Freezing extenders with antioxidants at three different doses led to lower percentages of acrosome and total sperm abnormalities, when compared to the controls ($P < 0.001$). However, the addition of 5mM inositol did not induce any difference in total sperm abnormalities, when compared to the controls. The antioxidants also did not show any effectiveness in the elimination of malondialdehyde (MDA) formation and the maintenance of glutathione peroxidase (GSH-PX) activity, when compared to the controls. Superoxide dismutase (SOD) activity was found to be higher in the presence of curcumin at all three dose levels and carnitine at 5mM, compared to the other groups. Glutathione (GSH) concentration was demonstrated to be maintained at a higher level with the addition of inositol, compared to the other groups. However, these differences in SOD and GSH levels were not significant, compared to the controls. All the antioxidants at all three dose levels resulted in a better protection of the sperm morphology (except for 5mMinositol with respect to the total sperm abnormalities), compared to the control samples. According to CASA, the best post-thawing sperm motility rate was recorded when the freezing extender was supplemented with 2.5mM curcumin. Further studies are required to obtain more conclusive results regarding the characterization of microscopic and oxidative stress parameters in cryopreserved goat sperm, using the different antioxidants.

497. Src Is a Signaling Molecule in Ouabain-Na^+K^+-ATPase-Stimulated Signaling Pathway During Bull Sperm Capacitation. Kalaivani Anpalakan, Mary M. Buhr, and Katie D. Hickey. University of Guelph, Guelph, ON, Canada; University of Saskatchewan, Saskatoon, SK, Canada

Na^+K^+-ATPase acts as a signaling molecule when ouabain binds, inducing dose-dependent protein tyrosine phosphorylation (p-tyr) during sperm capacitation. Src is present in head membranes of bull sperm and its expression alters during ouabain-induced p-tyr, so this study investigated Src's role in capacitation. Fresh bull sperm (n=3-6 ejaculates per trial) were pre-incubated with or without Src inhibitors herbimycin A (inhibits kinase) or SU6656 (prevents ATP binding to Src), followed by incubation in the presence or absence of ouabain or heparin. Extracted proteins were assessed by western blotting, band intensity measured, and quantities of each protein band compared by ANOVA. Both protein p-tyr and LPC-induced acrosome reaction increased ($p<0.05$) upon exposure of sperm to ouabain (242, 176, 81, 49 kDa) or heparin (176kDa). Inhibition of Src activity by herbimycin or SU6656 antagonized ($P<0.05$) various ouabain- and heparin-induced p-tyr, and herbimycin inhibited the ouabain-induced acrosome reaction, confirming Src's integral role in capacitation. Herbimycin actually reduced some Src itself ($p<0.05$) during ouabain- or heparin-induced capacitation incubation. However, since Src was activated by auto-phosphorylation in the presence of ouabain or heparin, it was of greater interest that herbimycin significantly reduced ouabain-enhanced Src autophosphorylation of the 59kDa phospho-Src and the other ouabain- (166-31kDa) and heparin-induced

dye induced abnormalities in the sperm cells. To assess sperm penetration abilities, spermatozoa (fresh: 2×10^5cells/100µl droplets; frozen: 5×10^5cells/100µl droplets) extended with treatment media were coincubated with conspecific in vitro matured oocytes for 18-20h. All presumptive embryos were fixed and stained three days post insemination to determine fertilization and cleavage rates based on DNA content. Morphology analysis confirmed radiographic dyes did not induce abnormalities in felid spermatozoa. Motility and forward progression were converted into sperm motility indices, and results demonstrated no significant differences between media for freshly collected spermatozoa. For frozen-thawed spermatozoa, a lower sperm motility index was calculated for Omnipaque vs control, but not compared with MD76. Motility index decreased over time for all treatments for both freshly collected and frozen-thawed sperm. Acrosomal integrity for freshly collected spermatozoa was significantly reduced when treated with Omnipaque vs MD76 but not compared with control media. Differences in acrosomal integrity were not seen within frozen-thawed treatment groups. Acrosomal integrity for freshly collected and frozen-thawed sperm cells also significantly decreased with time. Preliminary data also indicate sperm exposed to radiographic contrast media maintained their ability to penetrate and fertilize conspecific oocytes. These findings propose radiographic dye can be added to domestic cat spermatozoa with minimal reduction to sperm fitness, thus creating a foundation for developing improved transcervical insemination techniques for non-domestic felids.

499. Microarray-Based Analysis of Differential Expression of DNA Repair Genes During Spermatogenesis in the Mouse. Dipanwita Roy Choudhury, Eric de Waal, Chris Small, Michael D. Griswold, Christi A. Walter, and John R. McCarrey. University of Texas at San Antonio, San Antonio, TX, USA; Washington State University, Pullman, WA, USA; University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Spermatogenesis is the process by which male germ cells develop and differentiate to give rise to mature spermatozoa. The "Disposable Soma Theory" predicts that the integrity of the germline genome will be selectively maintained so that a pristine set of genetic information will be passed on to the next generation. A variety of individual DNA repair genes have been reported to be up-regulated in male germ cells compared to somatic tissues. The aim of the present analysis was to mine available microarray data characterizing gene expression during spermatogenesis to characterize expression of entire DNA repair pathways in 1) premeiotic type A spermatogonia, 2) meiotic pachytene spermatocytes and 3) postmeiotic round spermatids. We detected expression of 269 genes related to DNA repair in one or more of these cell types. These include genes involved in 12 different DNA pathways or gene groups including direct repair (15 genes), nucleotide excision repair (36 genes), base excision repair (26 genes), mismatch repair (21 genes), non-homologous end-joining (10 genes), homologous repair (23 genes), Fanconi anemia proteins (9 genes), check point factors (14 genes), translesion synthesis (11 genes), modulation of nucleotide pools (4 genes), DNA damage response (24 genes) and other factors