Abstracts

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of 20nM 20beta-S and 20nM OD 02-0. Treatment of croaker sperm with 10nM Wortmannin also prevented the activation of Akt, in the presence of 100nM 20beta-S or 02-0. Taken together, these results indicate an involvement of the PI3K/Akt pathway in mPRalpha mediated sperm hypermotility. To our knowledge, this is the first evidence that the PI3K/Akt pathway is involved in progestin-mediated sperm motility in a vertebrate species.

492. The Effect of Raffinose and Methionine on Frozen/Thawed Angora Buck (*Capra hircus ancryrensis*) Semen Quality, Lipid Peroxidation, and Antioxidant Enzyme Activities. Mustafa Numan Bucak, Purhan Barbaros Tuncer, Serpil Sariozkan, Ahmet Atessahin, Deniz Yeni, and Fatih Avdatek. Ministry of Agriculture and Rural Affairs, Lalahan, Turkey; Erciyes University, Kayseri, Turkey; Firat University, Elazig, Turkey; Afyon Kocatepe University, Ankara, Turkey

The aim of the present study was to determine the effects of different doses of raffinose and methionine on post-thawed semen quality, lipid peroxidation and antioxidant enzyme activities of Angora buck (Capra hircus ancryrensis) sperm following cryopreservation. Ejaculates collected from three Angora bucks were evaluated and pooled at 37°C. Semen samples, which were diluted with a Tris-based extender containing the additives raffinose (2.5, 5, 10 mM) and methionine (2.5, 5, 10 mM) and an extender containing no antioxidants (control), were cooled to 5oC and frozen in 0.25 ml French straws to be stored in liquid nitrogen. Frozen straws were thawed individually at 37°C for 20 sec in a water bath for evaluation. The freezing extender supplemented with 2.5 and 5 mM methionine led to higher percentages of computer–assisted sperm motility analysis (CASA) motility (63.6±7.0; 63.4±3.1 %, respectively), in comparison to the controls ($P<0.01$) following the freeze-thawing process. The addition of antioxidants did not provide any significant effect on the percentages of post-thaw subjective and CASA progressive motilities as well as sperm motion characteristics (VAP, VSL, LIN and ALH), compared to the control groups ($P>0.05$). The freezing extender with raffinose (5 and 10 mM) and methionine at three different doses (2.5, 5 and 10 mM) led to lower percentages of acrosome abnormalities, in comparison to the controls ($P<0.001$). In the comet test, raffinose (5 and 10 mM) and methionine (10 mM) gave scores lower than those of the controls, and thereby reduced DNA damage ($P<0.05$). Malondialdehyde (MDA) formation was found to be lower (1.8 ± 0.1 (nmol/L) in the group of 5 mM raffinose, compared to the controls following the freeze-thawing process ($P<0.01$). The additives did not show any effectiveness on the maintenance of SOD, GSH-PX and GSH activities, when compared to the controls ($P>0.05$). In conclusion, methionine and raffinose play a cryoprotective role against sperm CASA motility, acrosome abnormality and DNA damage. Raffinose 5 mM exhibited antioxidative properties, decreasing MDA levels. Further studies are required to obtain more concrete results on the characterization of microscopic parameters and antioxidant activities in cryopreserved goat sperm with different additives.

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 (13%), 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 decreased SOD activity when compared to the BME and control groups ($P < 0.001$).

494. Acrosome Reaction Induced by Zona Pellucida Is Mediated by Alpha-7 Nicotinic Acetylcholine and Epidermal Growth Factor Receptors. Yael Jaldety 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 (á7nAChR) leading to the trans-activation of epidermal-growth-factor-receptor (EGFR), calcium influx and the acrosome reaction. The data revealed that alpha7nAChR agonist initiates 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 tyrphostin (EGFR antagonist), suggesting that EGFR and the alpha7 subunit are activated by ZP. Furthermore, ZP or EGF did not induce the acrosome reaction in sperm from alpha7 null mice, suggesting that ZP-induced acrosome raction depends on the existance of sperm alpha7 and mediated by EGFR. Inhibition of alpha7 in wild type sperm revealed only partial inhibition of the acrosome reaction induced by EGF, supporting the idea that the alpha7nAChR is localized up-stream to the EGFR. Recent studies suggest that Src kinase can activate EGFR in sperm. It was also shown that Src co-localized with the alpha7 in human sperm. We showed here that Src inhibitor blocked the alpha7-dependent acrosome reaction and EGFR-phosphorylation suggesting that SRC-family mediate these processes. Moreover, EGFR phosphorylation on tyrosine-845 induced by ZP is also inhibited by SRC-family inhibitor suggesting that this kinase mediate the ZP-induced EGFR phosphorylation. It was recently shown in our lab that EGFR activation leads to calcium influx. Here we showed that agonist of the alpha7, EGFR or ZP induced calcium influx that leads to the acrosome reaction. However, in alpha7 null mice, these agonists did not induce calcium influx or acrosome reaction, and this blockade could be bypassed using calcium ionophore supporting the role of alpha7 in calcium influx induced by ZP. In conclusion we suggest that activation of alpha7 by ZP leads to SRC-family dependent-EGFR activation, Ca^{2+} influx and the occurrence of the acrosome reaction.

495. Suppression of Mitochondrial Activity in Activated Bovine Sperm Advances Capacitation Status and Induces Normal Embryogenesis. Yoku Kato, Rui Miyamoto, Janice L. Bailey, and Yoshikazu Nagao. Tokyo University of Agriculture and Technology, Tokyo, Japan; Utsunomiya University, Mohka, Japan; Universite Laval, Quebec City, QC, Canada

We have previously shown that activated bovine sperm induce capacitation. Activated sperm can fertilize normally. However, our previous study also indicated that the selection and injection (ICSI) of activated and hyperactivated sperm into bovine oocytes induced chromosomal aberration during early stages of development. Activation of sperm motility is a highly energetic process. Mitochondria produce significant amounts of ATP and reactive oxygen species (ROS) in those motile sperm. There is a possibility that ROS produced by activated sperm affect embryogenesis following sperm incorporation, resulting in chromosomal aberration. Here, we examined sperm capacitation state, ROS production along with the developmental ability of embryos following intracytoplasmic injection of activated sperm in which mitochondrial activity had been reduced experimentally. In the first experiment, we used a luminometer to investigate ROS production by sperm treated with carbonyl cyanide m-chlorophenyl hydrazone (cccp), an uncoupler of cell respiration. In our second experiment, mitochondria integrity and activity was determined quantitatively using mitotracker red and 5, 50, 6, 60-tetrachloro-1, 10, 3, 30-tetraethylbenzimidazolyl- carbocyanine iodide (JC-1) in groups of normal motile sperm (control group), activated (Act group) sperm, and activated sperm in which mitochondrial activity had been reduced by cccp (MT group). The proportion of 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