ReProForce

W o r k s h o p

"Mitochondria and reproduction"

Joint event of FP7 Project ReProForce

and COST Actions FA0602

2-3 June 2010

Institute of Biology and Immunology of Reproduction,
Bulgarian Academy of Sciences
Sofia, Bulgaria

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PROGRAM

Tuesday 1th June

10.00-18.00 Arrival of participants and hotel accomodation
19.00 Dinner

Wednesday 2nd June

Main topic: Mitochondrial role in the successfulness of reproductive process

9.30-10.30 Session 1

Chairpersons: M. Mollova, D. Kacheva, I. Shabalina, J. Ramalho
Opening and Welcome: M. Mollova, Coordinator of ReProForce
Information about ReProForce Project: M.Mollova, Coordinator of ReProForce
Information about FA0602: D.Kacheva, Member of MC COST Action FA0602

10.30-11.30 Session 2.

Significance of mitochondrial function for gametogenesis, quality of gametes and embryos

10.30-11.00 **Mitochondrial function in spermatogenesis and sperm function: implications for human reproduction.**
João Ramalho-Santos, PhD
(Center for Neuroscience and Cell Biology, Department of Life Sciences, University of Coimbra)

11.00-11.30 **Selenoproteins in mammalian spermatogenesis and male fertility.**
Carla Boitani, PhD
(Department of Histology and Medical Embryology, University of Rome "La Sapienza)

11.30-11.45 Coffee-break

Chairpersons: C. Boitani, R. Dumollard

11.45-12.15 **Suppressed spermatogenesis but Leydig cell hyperplasia in premature aging mtDNA-mutator mice**.
Irina Shabalina, MD, Ph
(Physiology Departmen, Wenner-Gren Institute, Stockholm University)

12.15-12.45 **Mitochondrial dysfunctions during cryopreservation and induced apoptosis in ram spermatozoa.**
M. Ivanova-Kicheva, A. Kukov, D. Daskalova.
(Institute of Biology and. Immunology of Reproduction-BAS, Sofia, Bulgaria)

12.45-13.15 Discussion

13.15-14.45 Lunch

14.45-15.15 **Mitochondrial and redox metabolism in the early mammalian embryo**.
R.Dumollard, PhD
(Laboratoire de Biologie du Développement, UMR 7009, Station Zoologique, France).

15.15-15.45 **Mitochondrial distribution and nitric oxide production in partially fragmented human embryo.**
Igor Golic
(University of Belgrade, Faculty of Biology)

15.45-16.15 Discussion

16.15-17.00 Work Group Meeting (FA0602)
17.00 Sofia tour
19.00 Dinner

Thursday 3th June

10.00-11.30 Session 3.

Effect of nutrition (bioactive food components, different diets) on the mitochondrial activity in reproductive tissues, gametes and embryos.

Chairpersons: M .Kicheva, M. Vyssokikh

10.00-10.30 **Consequences of Dietary Induced Chronic Hypothyroidism for the Reproductive Capacity of Male and Female offspring.**
Kathy Teerds, PhD
(Department of Animal Sciences, Wageningen University , the Netherlands)

10.30-11.00 **Beneficial effect of mitochondrial targeted plastoquinone on estrous cycle of premature aging mtDNA-mutator mice.**
Mikhail Vyssokikh, PhD
(Physiology Department, Wenner-Gren Institute, Stockholm University)

11.00-11.30 Discussion

11.30-11.45 Coffee break

11.45-13.00 Poster session

Chairpersons: J. Mihály, E. Kistanova

1. Comparison of the mitochondria activity isolated from testis and liver of rat.

E.Kistanova[1], Z. Drahota[2], J.Houstek[2], D.Kacheva[1]

([1]Institute of Biology and. Immunology of Reproduction-BAS, Sofia, Bulgaria, [2] Institute of Physiology, Academy of Sciences of Czech Republic)

2. Effect of selenopyran on the cytochrome C oxidase activity in mouse ovary and embryo outcome.

E. Kistanova[1], D. Kacheva[1], K. Shumkov[1], D. Abadjieva[1], G Borjaev[2,] M. Nevitov[2]

([1] Institute of Biology and. Immunoogy of Reproduction-BAS, Sofia, Bulgaria, [2]-Penza state agricultural academy, Russia)

3. Studies on NADH-tetrazolium reductase in ram sperm during short stock and cryopreservation.

R. Stefanov

(*Institute of Biology and. Immunoogy of Reproduction-BAS, Sofia, Bulgaria)*

4. **Influence of poly-unsaturated fatty acids, their mono-hydroylated metabolites and eicosanoids on gene expression of nuclear receptor specific target genes.**

Mihály Johanna

*(Department of Biochemistry and Molecular Biology, Faculty of medicine, University of Debrecen, Hungary***)**

5. Effect of dietary use of *Spirulina platensis* on the mitochondrial enzymes activity and ROS production in boar sperm.

Elena Kistanova[1], Yordan Marchev[2], Radka Nedeva[2] , Dimitrina Kacheva[1], Kiril Shumkov[1], Boyko Georgiev[1], Almantas Shimkus[3]

([1]Institute of Biology and Immunology of Reproduction-BAS, Sofia, Bulgaria [2]Agricultural Institute-Shumen, BAA, Bulgaria, [3]Lithuanian Veterinary Academy, Kaunas, Lithuania)

13.00-14.30 Lunch

14.30-16.00 Round table discussion

1. **Improvement of the reproductive properties: additional hormonal stimulation or proper diet with bioactive food components?**
2. **Could bioactive food components improve the reproductive health? If yes, by which way?**

Moderators: K. Teerds, M. Mourdjeva

16.00-16.20 **Closing remarks**: D. Kacheva, Member of MC COST Action FA0602

16.20- 17.30 Visit of institute laboratories. Visit of Anthropological museum.

PLENARY LECTURES ABSTRACTS

MITOCHONDRIAL FUNCTION IN SPERMATOGENESIS AND SPERM FUNCTION: IMPLICATIONS FOR HUMAN REPRODUCTION

João Ramalho-Santos
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Mitochondrial activity is crucial for gametogenesis, gamete function and embryogenesis, both in terms of ATP production via OXPHOS, ROS generation, calcium signalling, or the triggering of apoptosis. Indeed, mitochondrial defects are known to cause physiological dysfunction, including infertility. Metabolic shifts from mitochondria-produced ATP to glycolysis occur during gametogenesis, either reflecting developmental switches or substrate availability. Importantly, isolated testicular mitochondria have different bioenergetics' parameters when compared to other mitochondria normally used as predictive models (such as liver mitochondria) and respond differently to several substances, suggesting that testicular mitochondria might be a better model for toxicological studies in the reproductive system. On the other hand, although the exact role of sperm mitochondria is controversial, mitochondrial activity is a clear hallmark for sperm functionality, and can be used to predict reproductive success. In human samples mitochondrial activity correlates well with predicted reproductive success, being higher in samples from proven sperm donors, and lower in infertility patients. Within patients mitochondrial function seems also to be higher in patients with a normal sperm profile according to the WHO, versus highly impaired (oligoasthenoteratozoospermia-OAT) samples. Furthermore, in a single human sperm sample there are distinct sub-populations of cells with varying mitochondrial activity, as monitored using several specific fluorescent probes (Mitotracker Green, Mitotracker Red, JC-1). The separation of these sub-populations

using flow cytometry shows that sperm with enhanced mitochondrial function (Mito+) have more intact acrosomes, lower levels of DNA fragmentation and greater reproductive potential, as monitored by sperm decondensation and bovine oocyte activation assays. Flow cytometry separation of Mito+ seems to select functional sperm more specifically than the classic swim-up model. Interestingly, recent data suggests that mitochondrial activity can also modulate stem cell pluripotency as well as differentiation into distinct cellular fates.

SELENOPROTEINS IN MAMMALIAN SPERMATOGENESIS AND MALE FERTILITY

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In the mammalian testis, selenium is almost entirely associated with the enzyme Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx/GPx4), a selenoprotein belonging to the family of glutathione peroxidases. To date, three isoforms of GPx4 have been identified, having specific subcellular localization in mitochondria, cytosol and nuclei, respectively, and differing in their N-terminal amino acid sequence. It is commonly accepted that GPx4 behaves like a "moonlighting" protein, having different functions in spermatogenesis and spermatozoa, associated with specific intracellular localization. To gain more insight into mitochondrial (m) GPx4 function during male germ cell differentiation, we generated a transgenic mouse model, in which expression of the mGPx4 was targeted to the early meiotic cells (in which the endogenous selenoprotein levels are markedly lower than those of postmeiotic phase). Histological evaluation of mGPx4 overexpressing testes revealed a variable degree of germ cell apoptosis, seminiferous tubule degeneration and delayed differentiation. Furthermore, the impairment of the haploid phase of spermatogenesis was paralleled by a significant fertility reduction of adult mice. A

conclusive proof of the essential role of mGPx4 in sperm was recently provided by the specific disruption of this isoform. mGPx4 deficient sperm displayed severe structural abnormalities and male mGPx4 knockout mice were sterile. In line with this, a number of infertile men had sperm with impaired mGPx4 expression and activity.

As for the nuclear variant, nGPx4 first appears in haploid germ cells concomitantly with the initiation of chromatin condensation. It shows a peculiar localization in the nuclear matrix of round spermatids, elongated spermatids and epididymal sperm. When sperm isolated from wild type and nGPx4 knockout mice cauda epididymis were treated with a combination of heparin and glutathione to induce nuclear decondensation, KO sperm decondensed earlier than those of WT, demonstrating that the absence of nGPx4 in spermatozoa causes structural chromatin instability. This finding opens the possibility that nGPx4 is involved in the initial phases of early embryo development.

SUPPRESSED SPERMATOGENESIS BUT LEYDIG CELL HYPERPLASIA IN mt DNA MUTATOR MICE

Irina G. Shabalina[1], Louse Landreh[2], Natalia Gibanova[1], Irina Svechnikova[2], Alexandra Trifunovic[3], Olle Söder[2], Konstantin Svechnikov[2], Jan Nedergaard[1]

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MtDNA mutator mice expressing mtDNA polymerase with reduced activity (D257A point change mutation in the catalytic subunit) exhibit several features of premature aging, such as reduced lifespan, weight loss, reduced fat content, manifestation of alopecia, kyphosis and osteoporosis, anemia and reduced fertility (Trifunovic et al., 2004). Recently, we have shown that the observed phenotype in mtDNA

mutator mice is a direct consequence of the accumulation of mtDNA point mutations in protein-coding genes, leading to a decreased assembly of the respiratory chain complexes and thus to respiratory chain dysfunction (Edgar et al., 2009). The aim of the present study was to explore the testicular phenotype and the Leydig cell function of the mtDNA mutator mice. Histological analysis of the testes from 25-week-old mtDNA mutator mice revealed a complete disruption of spermatogenesis, associated with a degeneration of germ cells. In contrast to germ cells, Leydig cells exhibited hyperplasia. Production of testosterone under basal conditions in vitro was enhanced, while the responsiveness to stimulation by hCG and (Bu)2 cAMP was unchanged. Similarly to other tissues (liver, heart, skeletal muscle), Leydig cells of mtDNA mutator mice were extremely deficient in the content of mitochondrial respiratory chain complex IV, whereas subunits of FoF1-ATP-ase were not affected. Interestingly, Leydig cells of mtDNA mutant mice were significantly more active (4-fold wild-type) in the extracellular reduction of WST-1 into formazan, suggesting the existence of high redox potential and/or superoxide production of non-mitochondrial nature in mtDNA mutator mice. Thus, mtDNA mutator mice are valuable tool for evaluation of a mitochondrial role in male (in)fertility and steroidogenesis.

MITOCHONDIA DISFUNCTION DURING CRYOPRESERVATION AND INDUCED APOPTOSIS IN RAM SPERMATOZOA

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The behaviour of mitochondria and the connection with membrane phosphatidylserine (PS) molecules externalisation from spermatozoa after cryopreservation, UV illumination (apoptosis induced factor) and capacitation (Ca++, HCO3-, and albumin – control samples), was

investigated. After cryopreservation, UV irradiation and capactation of sperm cells the mitochondrial transmembrane potential meseared by R123 showed differences, which are connected with the specific translocation of membrane PS from the inner to the outer plasma membrane (PM) monolayer. After cryopreservation and UV irradiation membrane PS translocation was localized mainly in the region of midpiece or the whole PM was affected, while after capacitation PS scrambling occurred mainly at the apical head region, where specific clusters were visualized. Also, an interesting spiral arrangement of aggregates consisting of membrane PS molecules in the mid-piece (where the mitochondria are located) was observed. The quality (motility and survival rate) of these spermatozoa was significantly lower, compared to capacitated sperm cells. It can be pointed out that sperm cells with pour functional characteristics showed lower intact mitochondria and a specific PS externalization in the midpiece region, which is different from the PS scrambling observed in viable, capacitated spermatozoa, where the functional mitochondria predominated. In conclusion after cryopreservation and UV illumination a specific externalization of PS molecules in the region of mitochondria is connected with intracellular signalling leading to disturbance of sperm functionality.

MITOCHONDRIAL AND REDOX METABOLISM IN THE EARLY MAMMALIAN EMBRYO

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The metabolism of mammalian oocytes and early embryos is transformed during ovulation and at fertilization. From ovulation up to implantation the oocyte and embryo rely solely on internal

metabolism to supply all energy demand and to set the intracellular redox state. At fertilization, sperm entry triggers intracellular calcium signals that stimulate both embryonic development and the ATP production required to support such development. ATP production in the oocyte and early embryo is exclusively supplied by mitochondrial oxidative phosphorylation. A by-product of oxidative metabolism is the generation of deleterious reactive oxygen species (ROS) to which embryos are very sensitive. By monitoring cytosolic and mitochondrial calcium and ATP levels together with the intracellular redox state, we assessed the metabolism of single oocyte or embryo at fertilization and during pre-implantation development. We found that ATP supply and demand are closely coupled in early embryos to allow for a minimal stimulation of mitochondrial oxidative metabolism in order to minimize ROS production. Furthermore, we characterized the metabolism of the major metabolic substrates present in culture media and found that it was very specific with exogenous pyruvate being the main energetic substrate whereas glucose is poorly metabolised and lactate-derived pyruvate is not metabolised by mitochondria. The importance of such critically balanced metabolism is reflected in the high sensitivity of early embryos to metabolic stress (generated internally or from environmental insults) and led to the discovery that oocytes and early embryos with a "quieter" metabolism have a much better developmental potential. Indeed, it was recently found that not all mitochondria present in the egg are necessary for early development and these mitochondria thus seemed stored for later development. During cleavage stages, metabolism may be assayed non-invasively by measuring oxygen consumption, intracellular redox state or by amino-acid turnover profiling. These studies have all concluded that embryos showing the lower metabolic activity have a better developmental potential probably because they can better circumvent intracellular oxidation arising from aerobic metabolism.

MITOCHONDRIAL DISTRIBUTION AND NITRIC OXIDE PRODUCTION IN PARTIALLY FRAGMENTED HUMAN EMBRYO

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Mitochondria play a primary role in cellular energetic metabolism, homeostasis, and death. They are the most abundant organelles in mammalian oocyte and directly involved at several levels in the reproductive process. Their functional status influences the quality of oocytes and contributes to the fertilization and embryonic development. The localization of mitochondria in the egg during maturation and their segregation to blastomeres in the cleaving embryos are strictly regulated. Nitric oxide (NO) is considered to be involved in mitochondriogenesis and mitochondrial remodeling. Previous studies have demonstrated its role in regulation of preimplantation mouse embryo development. In this study, we examined mitochondrial population in the partially fragmented preimplantation human embryos. Mitochondrial remodeling and distribution was assessed with MitoTracker Green using confocal microscopy, while intracellular NO has been investigated by using the fluorescent indicator 4,5-diaminofluorescein-2 diacetate. In good quality embryos, the production of NO was uniform in all blastomeres. MitoTracker staining revealed the asymmetric and perinuclear mitochondrial distribution. The enhanced NO production detected in fragments may be involved in the process of cell death in targeted blastomeres of the preimplantation embryo. Partial fragmentation was commonly seen, and certain ultrastructural mitochondrial changes were also noted. The obtained results suggest that blastomeres of partially fragmented preimplantation human embryos are characterized by impaired production of nitric oxide and mitochondrial distribution.

Seventh Framework Programme

EFFECTS OF CHRONIC PRENATAL/POSTNATAL HYPOTHYROIDISM ON GONADAL DEVELOPMENT AND FERTILITY IN MALE AND FEMALE RATS

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Alterations in thyroid hormone levels are well known to influence key functions in growth and development. The trace element iodide is essential for adequate thyroid hormone synthesis. Decreased uptake of this nutrient usually results in elevated levels of thyroid stimulating hormone (TSH) due to a reduced negative feedback of thyroid hormone on the pituitary release of TSH. The latter leads to deficiency disorders like goiter, decreased fertility and retarded physical and mental development. Although in many countries food products are fortified with iodide, still, not all humans and animals have access to or use these fortified products. For example 15-20% of women of childbearing age in the USA have a suboptimal iodide intake.

A dietary approach was used to induce a relatively mild form of hypothyroidism in rats during fetal development to investigate the effects on testicular and ovarian development. Dams were fed either an iodide-poor diet to which 0.5% perchlorate was added to deplete endogenous iodide stores, or received a control diet (iodide content according to the AIN-93 guidelines). The hypothyroid diet was continued until the animals were sacrificed between days 16 and 84 postpartum (pp).

Under hypothyroid conditions Sertoli cells continue to proliferate up to at least 28 days pp and tubule lumen formation is first completed by day 42 pp, suggesting a delay in Sertoli cell differentiation. At the same time spermatogenesis did not proceed past meiosis, although plasma testosterone levels were 8- to 10-fold higher

during this period compared to the age-matched controls. Next, we determined the presence of androgen receptors (AR) in the testis by immunohistochemistry. Strikingly, in the 16 day-old hypothyroid testis no AR protein could be detected in the Sertoli cells, while in peritubular/myoid cells the expression seemed unaffected. The absence in AR signaling was confirmed by the continued presence of anti-Müllerian hormone (AMH) protein in the cytoplasm of the Sertoli cells up to 35 days pp. Between the age of 21 and 35 days pp. Sertoli cells acquired AR immunoreactivity and meiosis started to progress. By day 42 round spermatids were detected and by day 50 pp elongated spermatids were frequently observed in the hypothyroid testis. By 84 days pp spermatogenesis in the hypothyroid rats had completely normalized, testis weight and tubule diameter were identical with the control animals. The results of this study strongly suggest that chronic hypothyroidism delays the appearance of the AR in Sertoli cells, thus transiently inhibiting spermatogenesis.

In the hypothyroid female offspring the situation was different. Although the bodyweight of the females was severely reduced compared to the age-matched controls, the animals entered puberty at the same age as the controls, around day 35 pp. Investigation of plasma leptin levels and total body fat content showed that at the ages of 21 and 28 days pp. plasma leptin levels were significantly elevated in the hypothyroid females, while total body fat content was elevated at the ages of 28 and 35 days pp. compared to the age-matched controls. These results show that despite the reduction in body weight, body fat mass in hypothyroid female rats is increased and further stress the importance of leptin for the induction of puberty in female rats.

Follicular development was investigated in 64-day-old female rats. In the hypothyroid rats the number of preantral and antral follicles per ovary was significantly decreased compared to the euthyroid controls. Concomitantly, the number of corpora lutea was reduced, suggesting that up to the age of 64 days less follicles had ovulated from the hypothyroid ovaries. Preliminary data suggest that under hypothyroid

conditions the number of degenerating follicles is increased. At present we plan to expand our preliminary data on follicle numbers and atresia under hypothyroid conditions and to investigate the cause of the increased follicular degeneration. It is very well possible that under hypothyroid conditions the viability of the oocytes is affected. One way by which cell viability can be affected is by mitochondrial dysfunction. We therefore plan to investigate whether indeed mitochondrial dysfunction plays a role in the observed decrease in healthy follicles under hypothyroid conditions.

Despite the reduced numbers of healthy follicles, chronic hypothyroid rats are fertile. Breeding experiments in which hypothyroid males were mated with hypothyroid females resulted in the birth of viable offspring. Although the number of animals included in this experiment was small, the mating success was 83%, the pregnancy rate 80% and the life birth rate 75%. However, the litter size was significantly reduced under hypothyroid conditions.

Taken together, chronic prenatal/postnatal hypothyroidism does affect both male and female gonadal development, but does not result in infertility.

BENEFICIAL EFFECT OF MITOCHONDRIAL TARGETED PLASTOQUINONE ON ESTROUS CYCLE IN PREMATURE AGEING MTDNA MUTATOR MICE

Mikhail Yu. Vyssokikh[1,2], Irina G. Shabalina[1], Zinaida Rozhdestvenskaya[1], Antonina Pustovidko[2], Natalia Gibanova[1], Alexandra Trifunovic[3], Jan Nedergaard[1], Vladimir P. Skulachev[2], Barbara Cannon[1]

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A new type of mitochondrial-targeted compounds (SkQs) - consisting of plastoquinone (an antioxidant moiety), triphenylphosphonium (a penetrating cation), and a decane linker - has been suggested as a potential tool for treatment of senescence and age-related diseases (Skulachev et al., 2009). MtDNA mutator mice expressing mtDNA polymerase with reduced proof-reading activity exhibit several features of premature aging, such as reduced lifespan, weight loss, reduced fat content, manifestation of alopecia, kyphosis and osteoporosis, anemia and reduced male fertility (Trifunovic et al., 2004). Among these other markers of accelerated senescence, mtDNA mutator mice also have impaired estrous cycle with irregularity and premature stop of appearance of estrus as compared to wild type mice. We have treated females mtDNA mutator and wild type mice with SkQ added to the drinking water (1.0 μmol/day x kg body weight). SkQ1 treatment increased the number of estruses and the regularity of the estrous cycle in mtDNA Mutator mice. The antioxidant activity of SkQ was revealed as delayed spontaneous formation of MDA, a lowered content of endogenously formed HNE-adducts, and recovered levels of endogenous cardiolipin. These features may at least in part explain the beneficial effect of SkQ. Thus, mitochondrial targeted plastoquinone may be a suggestion for a novel pharmacological treatment of premature ageing and mitochondrial diseases.

POSTERS ' ABSTRACTS

THE COMPARISON OF THE MITOCHONDRIA ACTIVITY ISOLATED FROM TESTIS AND LIVER OF RAT.

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It is known that mitochondrial structure, distribution and metabolic activity are tissue depend. The specific mitochondrial activity in testes originates from their double function - spermatogenesis and Leydig cells steroidogenesis as well as in liver from high metabolic activity of the organ.

The aim of the present work was the comparison of the liver and testes mitochondrial abilities to utilize the metabolic substrates measured by oxygen consumption. Mitochondria were isolated from liver and testis of male rats according the standard procedure at 0–4C° by differential centrifugation and washing of the mitochondrial pellet three times in the medium. Oxygen consumption of mitochondria was measured with a High Resolution Oxygraph (Oroboros, Austria) in 2 ml of incubation medium. The different substrates - succinate, glycerol phosphate, (GP) glytamate and malate were added to start the reaction. Also the effect of respiratory chain inhibitors such KCN and antimycin A (AA) was studied. Oxygen uptake was expressed as pmol oxygen/s/mg mitochondrial protein.

The results showed a higher total oxygen consumption of rat mitochondria in liver than in testes. The substrates such succinate, glytamate and malate were oxidized actively by liver mitochondria. In contrast, testes mitochondria oxidize better the GP substrate. This process activates in the presence of ADP (109,3 and 210,3 pmol oxygen/s/mg protein after ADP addition). High mitochondrial GP oxidation indicates high expression of mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH). We observed the glycerol phosphate depended ROS generation in testes mitochondria. This production was highly stimulated by a electron acceptor ferricyanide (FeCN). FeCN-induced oxygen uptake increased in the presence of respiratory chain inhibitors as KCN and antimycin A. The COX activity measured by following oxygen consumption in the presence of cytochrome c and tetramethyl-p-phenyldiamine (TMPD)/ascorbate as the electron donor was higher in testes mitochondria than in liver.

EFFECT OF SELENOPYRAN ON THE ACTIVITY OF CYTOCHROME C OXIDASE IN MOUSE OVARIES AND EMBRYO OUTCOME

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The effect of biological active substance, selenium organic compound - Selenopyran obtained from female animals before ovulation, on the activity of cytochrome C oxidase in mouse ovaries and on the number and quality of the in vivo produced embryos was studied. The experiment was carried out with 20 laboratory white mice at reproductive age. During the 30 days the animals of the experimental group were injected intraperitonealy (i.p.) at each 10-th day with oil solution of the selenopyran in dose 100 μg/kg live weight. The mitochondrial function of ovaries by activity of cytochrome C oxidase (COX) in homogenates obtained from ovaries was investigated. After superovulation and insemination by standard protocol, the embryos were recovered, estimated and counted. Also the physiological parameters of blood as a total protein, glucose content, selenium level and activity of GOT and GPT were observed.

Treating of female animals with selenium organic compound before ovulation increases the embryo production in vivo. The highest total number of embryos was observed in group treated with selenopyran (27,8 against 20 in control group). This data correspond with the results reflected the activity of the mitochondrial enzyme cytohrome c oxidase in ovaries. The highest increase of it was observed in experimental group, too ($P<0,05$). Probably, the effect of selenopyran is realized by the activation of the metabolic processes in the ovaries reflected on the quantity and quality of ovulated oocytes and as a result of that on the embryo production.

Seventh Framework Programme

STUDIES ON NADH-TETRAZOLIUM REDUCTASE IN RAM SPERM DURING SHORT STOCK AND CRYOPRESERVATION

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It was established that the catabolic processes are predominant over the anabolic in the spermatozoa. Essential role on the control of the transformation of energy of male gametes play the ratio between nicotinamide dinucleotide (NAD) and nicotinamide dinucleotide phosphate (NADPH). The group of enzymes referred to this which despite belonging to the different metabolic cycles catalyzes oxide-reduction reaction, contributing to the maintenance of necessary intracellular balance and the energy is released for the cell metabolism. The enzyme system NADH- tetrazolium reductase can be attached to them which were used as marker for the functional activity of sperm mitochondrion.

The aim of this study is the activity determination of NADH-tetrazolium reductase enzyme system in ram sperm during short stock and cryopreservation in relation with their viability. The experiments included 14 ejaculates collected by artificial vagina from 3 rams (2 years old), placed on the same condition of food, breeding and sexual use, conformed to the norms. The ejaculates were split into 4 parts, diluted in the ration1+3, as the first (A and B) uses the medium for the comparison at 4°C and the seconds(C and D) - the medium for cryopreservation. The samples from the groups A and C included the ejaculates with motility 65-70 %, and the groups B and D those with motility over 70%. It was established, that the fresh ram ejaculates with lower motility (below 70%) as and after their cryopreservation have values referred to the motility after incubation at 39°C for 300min that are significantly lower ($p<0.01$, $p<0.01$).The spermatozoa with lower motility during collection of semen (below 70%), after short stock

and cryopreservation possess lower values of mean cytochemical coefficient for NADPH tetrazolium reductase activity ($P<0.5$, $P<0.5$).

The values of mean cytochemical coefficient for NADPH tetrazolium reductase activity of spermatozoa can be used as criterion for the dynamic of metabolic processes and the viability of ram sperm.

INFLUENCE OF POLY-UNSATURATED FATTY ACIDS, THEIR MONO-HYDROXYLATED METABOLITES AND EICOSANOIDS ON GENE EXPRESSION OF NUCLEAR RECEPTOR SPECIFIC TARGET GENES

Johanna Mihály, Ralph Rühl
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Poly-unsaturated fatty acids (PUFAs), like linoleic acid, eicosapentaenoic acid and arachidonic acid are essential nutrients and possible nutritional relevant activators of nuclear hormone receptors like retinoid X receptor (RXR), peroxisome proliferator-activated receptor (PPAR) α/β/γ and retinoic acid receptor (RAR). Hydroxyeicosatetraenoic acid (HETE), hydroxyeicosapentaenoic acid (HEPE) and hydroxyoctadienoic acid (HODE) as the mono-hydroxylated metabolites, respectively the prostaglandin (PGD2, PGI2, PGJ2, 15-deoxy 12,14 PGJ2) and leukotriene (LTB4) derivatives of these PUFAs are known to be more potent activators of PPARs. These fatty acids and their mono-hydroxylated metabolites were incubated on MM6 cells (human monocytes) in a concentration range from 10-6-10-9M and the gene expression of nuclear receptor specific target genes like the fatty acid binding protein (FABP4) and adipocyte differenciation related protein (ADRP) as PPAR target genes and transglutaminase (TG2) as an RAR/RXR target gene were determined by RT-QPCR. After the application of physiologically / nutritionally relevant concentrations

of PUFAs, respectively HETEs and HODEs we could determine only low transcriptional activation of the nuclear hormone receptor target genes in comparison to the highly potent synthetic agonists. Co-administrations of PUFA metabolites with RXR agonists significantly modify the gene expression. Our results show that 5-HETE is down-regulating PPAR response genes; LTB4, 8-HETE and 15-deoxy 12,14 PGJ2 are down-regulating RAR response genes and 15-deoxy 12,14 PGJ2, PGD2, PGI2 and PGJ2 are up-regulating PPAR response genes. From these experiments we postulate that low concentrations of PPAR activators are sufficient to alter PPAR relevant target gene activation when an RXR ligand is present.

EFFECT OF DIETARY USE OF SPIRULINA PLATENSIS ON THE MITOCHONDRIAL ENZYMES ACTIVITY AND ROS PRODUCTION IN BOAR SPERM

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Microalgae Spirulina platensis contents a lot of vitally important for the organisms minerals and macroelements such as iron, calcium, sodium, potassium, copper, magnesium, phosphorus, selenium, vitamins, carotine, nucleic acids, enzymes and other active substances due it a value feed additive for the agricultural animals.

The aim of this work was studying the effect of Spirulina platensis on the mitochondrial activity of boar sperm. The experiment was carried out with 6 boars from Danube white breed in the experimental animal base of the Agricultural Institute - Shumen. The time of the experiment was divided in the control and experimental periods. During the

control period the animals received the main diet in accordance with Bulgarian state standard BDS- 1642-96. In the experimental period to the main diet were added 7 ml/ per head the fresh biomass of microalgae preserved by melasa (final quantity - 1,4 mg Spirulina platensis). The total dehydrogenase activity (DH) of spermatozoids was estimated by methylene-blue reduction method. The activity of the spermatozoids' lactate dehydrogenase (LDH) in the water and tritons' extracts was estimated by the spectrophotometric method of Wroblewski and LaDue (1955) after centrifugation and removing of sperm plasma. The evaluation of the ability of spermatozoa to produce reactive oxygen species (ROS) was done by using nitroblue tetrazolium (NBT) staining (Navid et all, 2003).

The obtained results showed a higher activity of total DH as well as LDH in the spermatozoa of boars received Spirulina. These spermatozoa demonstrated better survivability during the storage at temperature above zero (15 ºC) and thermal resistance at 39ºC. The antioxidative properties of Spirulina reduce semen oxidative stress. The NTB test pointed at a lower ROS production in sperm during the post treatment period..