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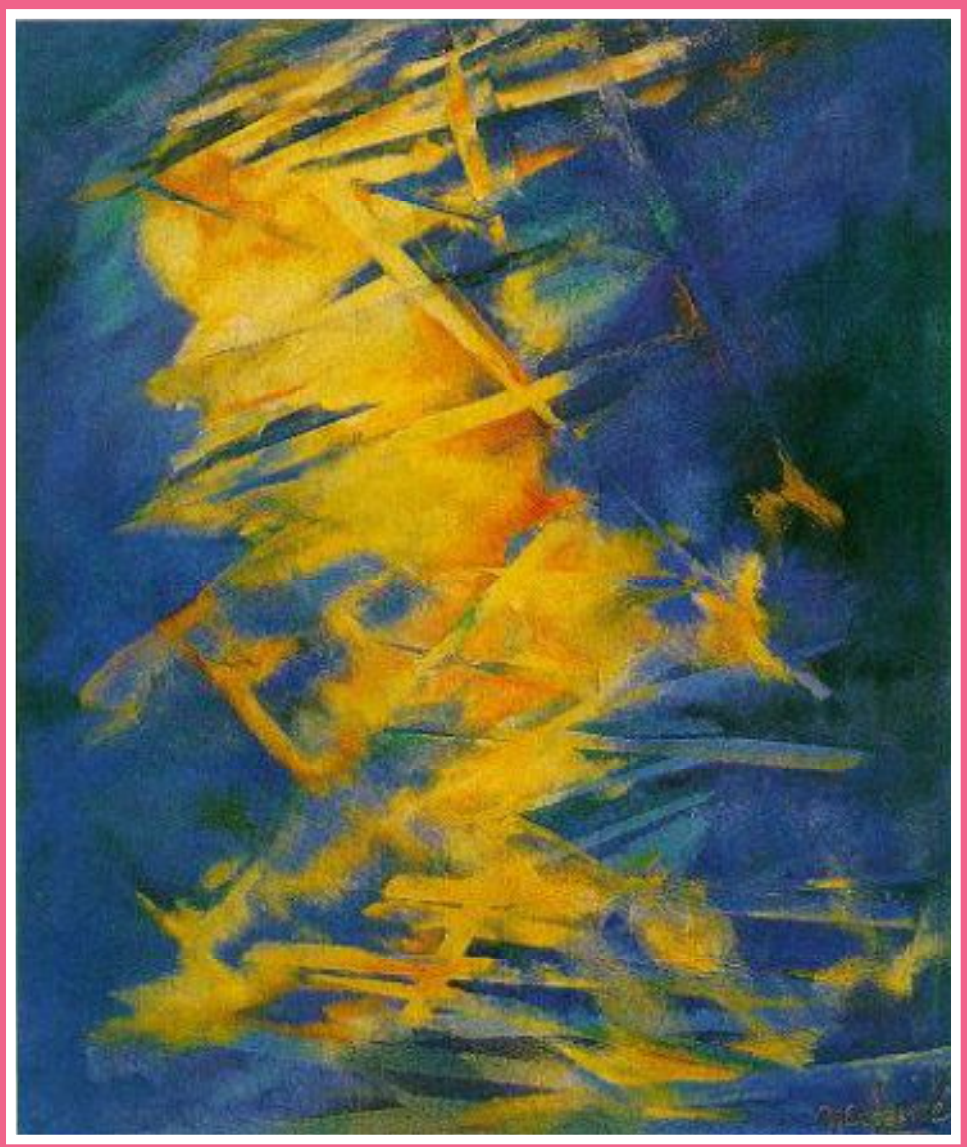

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pressure (BP) was recorded; after 10w of treatment rats were euthanized and hearts obtained. BP, LVH index and cTRH expression were increased in SHR vs WKY. VPA slightly attenuated (ANOVA,$p<0.05$) the higher BP (mmHg) seen in untreated SHR, without effect in WKY (**WKY**= C:128±4 vs VPA:126±3 and **SHR**=C220±4 vs VPA201±4). Hypertrophic index (HW/BW*100) was reduced ($p<0.05$) only in SHR (C:0.4516±0.02 vs VPA:0.3950±0.02). By ecocardiography we found a ($p<0.05$) reduction in LVPWT(mm) only in SHR (C:0.310±0.02 vs VPA:0.242±0.021). VPA normalizes ($p<0.05$) the higher expression of BNP and type 3 collagen in the LV of SHR indicating a strong reduction in fibrosis. This effect was confirmed by Masson's Trichrome and Sirius Red stainings ($p<0.01$). The higher TRH mRNA in SHR heart was reduce in the SHR+VPA to values similar to WKY (**WKY,**C:0.61±0.7vs VPA:0.41±0.97; **SHR,**C:5.72+0.9 vs VPA:0.61+0.9, $p<0.05$). Decreased TRH level by IHQ induced by HDAC inhibition confirms this result. Offspring born from VPA-treated parents with a 2-weeks washout period before mating, and which did not receive VPA ever, had a reduction of hypertrophy, fibrosis and cardiacTRH expression showing transgenerational inheritance. We described for the first time that VPA reduces fibrosis in an independent manner of LVH, effects inherited by the next generation. Our results strongly suggest that epigenetic TRH modulation may play a role.

REPRODUCTION AND FERTILITY 1

(772) AGING-ASSOCIATED INFLAMMATION IN TESTES OF SYRIAN HAMSTERS

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Abstract: Aging constitutes a universal, multi-factorial, progressive and irreversible process. Although the aging process may vary among different tissues, it is usually associated to a chronic inflammatory condition. Relevant to male reproductive aging, we have previously shown an inverse association between longevity and testicular pro-inflammatory state in mouse models with delayed (Ames dwarf Prop1$^{-/-}$ and growth hormone releasing hormone-knockout mice) or accelerated (growth hormone-transgenic mice) aging.

In this study, we analyzed testicular aging in a physiological animal model. To this aim, testes from young adults (5m: 5 months) and aged Syrian hamsters (20m-22m: 20-22 months) were used.

Immunohistochemical studies confirmed an aged-related significant increase in total numbers of Iba1-immunoreactive testicular macrophages (MAC) in which interstitial MACs solely accounted for the rise (Total testicular MAC/mm^2, 5m: 66.28±6.67^a; 20m: 110.00±10.00^b; 22m: 120.50±14.50^b; Mean±SEM, $p<0.05$). Testicular mRNA expression of cytokine IL1β, one of the main secretory products of MACs during inflammation, was found to be significantly higher in aged testes (5m: 1.00±0.05^a; 20m: 11.29±2.58^b; 22m: 16.56±1.80^b; $p<0.05$). Moreover, mRNA expression of cyclooxygenase 2 (COX2), a key enzyme in prostaglandin synthesis and a clear marker of inflammation, was also induced in an aged-dependent manner (5m: 1.00±0.16^a; 20m: 4.74±1.04^b; 22m: 3.47±0.96^b; $p<0.05$). A similar tendency was seen when testicular protein levels of IL1β and COX2 were evaluated.

Collectively these data suggest the development of a pro-inflammatory profile during physiological reproductive aging in the hamster testis.

Keywords: testis, aging, inflammation, COX2, IL1β

(508) ENZYMES INVOLVED IN THE BIOSYNTHESIS OF THE VERY-LONG-CHAIN POLYUNSATURATED FATTY ACIDS OF RAT SPERMATOGENIC CELL SPHINGOLIPIDS

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The sphingolipids (SL) of rodent germ cells contain very-long-chain polyunsaturated fatty acids (V), in nonhydroxy (n-V) and 2-hydroxy (h-V) forms, whose biosynthesis requires the expression of several elongases (Elovl) and a fatty acid 2-hydroxylase (Fa2h). Our objective was to characterize the expression of these enzymes as a function of postnatal development and germ cell differentiation. We employed qPCR for mRNA levels, Western blot and immunofluorescence for protein expression, and [^{3}H]-labeled precursors for enzyme activity. Elovl4, involved in C_{24}-C_{34} fatty acid biosynthesis, was expressed at the mRNA, but not at the protein level, at early prepuberal ages. Such mRNA was a product of Sertoli cells. Elovl4 mRNA *and* protein were both produced by germ cells. In agreement with the relative abundance of n-V in their SL, the Elovl4 enzymatic activity was higher in the premeiotic pachytene spermatocytes than in postmeiotic round and late spermatids ($p<0.05$), and was negligible in Sertoli cells. By contrast to *Elovl4* mRNA, that of *Fa2h* was absent from Sertoli cells and from prepuberal testes ($p<0.05$). *Fa2h* mRNA *and* protein were detectable only in concomitance with the appearance of spermatids, the sperm precursors whose SL are the richest in h-V. Consistently, selective depletion of germ (but not Sertoli) cells from adult rat testes by exposures to mild hyperthermia reduced the mRNA levels of *Fa2h* to a much larger extent ($p<0.001$) than that of *Elovl4.* Among germ cells of adult testes, Elovl4 protein content was high in spermatocytes and late spermatids, while Fa2h was mostly expressed in the latter, residual bodies and spermatozoa. By using inhibitors of specific steps of the SL biosynthetic route, germ cells in culture showed ability to *de novo* synthesize SL containing n-V and h-V. Our results underscore the presence of a developmentally programmed and a cell-specific regulation of the Elovl4 and Fa2h expression and activity as germ cell differentiation proceeds.

Keywords: spermatogenic cells, sphingolipids, very-long-chain PUFA

(1600) NONYLPHENOL INDUCES CYTOSKELETAL CHANGES AND RELEASE OF PROINFLAMMATORY MEDIATORS IN RAT SERTOLI CELLS *IN VITRO*

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Nonylphenol (NP), an alkylphenol present in plasticizers, is an endocrine disrupting chemical that is potentially dangerous for male reproduction in mammals including man. Because Sertoli cells (SC) provide structural and metabolic support to germ cells, in this study the hypothesis that exposures of SC to NP affect their metabolic functions and the production of bioactive molecules was evaluated. Primary cultures of SC were preincubated with [^{3}H]arachidonic acid (AA) to label their lipids and then were treated with NP. The NP exposures resulted in increased concentrations of free AA in cells and medium, indicating that such AA was released from [^{3}H]-labeled lipids ($p<0.01$). This lipid was mostly phosphatidylinositol, acted upon after activation of a protein kinase A (PKA)/cytoplasmic phospholipase A2 (cPLA2). In NP-exposed SC, an increase of diacylglycerols (DAG) also took place in both, cells and medium ($p<0.01$). Part of such DAG may have served as second messengers, since NP-increased DAG were associated with an augmented production of PGE2 and expression (mRNA) of COX2. Since the network of vimentin intermediate filaments is important for intracellular lipid transport, the effects of NP on the structure of this network in relation to the formation of cytoplasmic lipid droplets (LD) was studied. In NP-treated SC, the vimentin network was redistributed and the LD size was increased. The NP-dependent cytoskeletal redistribution was prevented by preincubation with H89, a PKA inhibitor. The formation of large LD was prevented by preincubation with either H89 or MEP, a PLA2 inhibitor, suggesting the participation of PKA and cPLA2 in LD biogenesis. We conclude that NP is involved in