experimental groups were found (F(2,21)=0.18, P=0.83). Conclusions: these results indicate that bending sperm immaturity in PUFA ω3 deficient mice is not related to higher ROS production and does not affect in vivo fertilization capability. Further investigations are needed to better understand the relevance of dietary PUFA ω3 in male fertility.

539. (278) **HYPEROSMOLARITY INDUCES CAVEOLAE INTERNALIZATION IMPAIRING EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION**

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During placentation, human extravillous trophoblast (EVT) cells need to proliferate, migrate, and differentiate correctly to ensure proper placental development. Previously, we reported that caveolae are required for the proper migration and endovascular differentiation of EVT. Recently, we found that hyperosmolarity alters EVT cell migration and invasion. However, up to now, the molecular mechanism is unknown. We hypothesized that hyperosmolarity increases caveolae endocytosis and caveolin-1 (Cav-1) degradation, altering EVT cell differentiation.

Objectives: Our aim was to explore the effect of hyperosmolarity on caveolae microdomains and the impact on the EVT cell differentiation.

Methods: The human EVT Swan-71 cell line was cultured in complete DMEM/F-12. 100 mM of sucrose was added to generate the hyperosmolar condition. Cell viability was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The localization of caveolae was analyzed by Transmission Electron Microscopy (TEM). Cav-1 expression was determined by WB in different conditions (isoosmolarity or control and hyperosmolarity, with or without MG-132- a proteasome inhibitor- and NH_4Cl- a lysosomal inhibitor). Endovascular differentiation was analyzed by the formation of tube-like structures in plates coated with Matrigel®.

Results: Cell viability was not affected by the experimental conditions. TEM showed that hyperosmolarity induced the internalization of caveolae. In addition, hyperosmolarity also increased Cav-1 protein degradation by lysosomal proteolysis ($p<0.05$, n=3) and significantly reduced the formation of tube-like structures compared to control ($p<0.05$, n=4).

Conclusion: Our results show that hyperosmolarity leads to the internalization of caveolae and the degradation of Cav-1, impairing endovascular differentiation of EVT cells.

540. (362) **THE ROLE OF AQP3 IN AMNION CELLS EXPOSED TO AN OSMOTIC STRESS**

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INTRODUCTION: AQPs in fetal membranes have been proposed to regulate the amniotic fluid volume. Altered expression of these proteins might be associated with oligo and polyhydramnios syndromes. However, we recently observed that the blocking of AQP3 did not prevent cell swelling in amnion cells. In addition, under osmotic stress the pattern expression of amnion AQP3 was different from other AQPs, suggesting a different role for this protein.

OBJETIVE: To study the regulation of AQP3 and its role in the amnion.

METHODS: Amnion-derived WISH cells were cultured in hypo (150 mOsm) and hyperosmolar (400 mOsm) conditions. Levels of phosphorylated ERK (pERK), JUNK (pJNK) and p38 (p-p38) were studied. Nf-κB and tonEBP expressions were assessed in nuclear and cytosolic fractions. AQP3 expression was analyzed after the inhibition of Nf-κB and tonEBP pathways with Sodium Salicylate and Cyclosporine-A, respectively. Cell viability was studied by MTT assay. Apoptosis was studied by TUNEL assay and Bax/Bcl-2 ratio after the inhibition of AQP3 using $CuSO_4$ or the specific siRNA.

RESULTS: pERK levels increased in hyperosmolarity and did not change in hypoosmolarity ($p<0.001$; n=6). No significant differences were observed in p-p38 and pJNK (ns; n=6). Nf-κB and tonEBP expressions increased in nuclear fraction only in hyperosmolarity ($p<0.05$; n=5; $p<0.01$; n=5). In this condition, the blocking of Nf-κB pathway increased AQP3 expression ($p<0.001$; n=5) compared to controls, while the inhibition of tonEBP pathway did not modify its expression. Regarding cell viability in hiperosmolarity, the blocking of AQP3 decreased MTT incorporation ($p<0.01$; n=8). Moreover, Bax/Bcl-2 ratio and the number of apoptotic nuclei increased after $CuSO_4$ treatment ($p<0.001$; n=5; $p<0.001$; n=9) and AQP3 silencing ($p<0.05$; n=5; $p<0.01$; n=10).

CONCLUSION: Our findings suggest that AQP3 may have an important role in the survival of the amniotic cells and its expression may be regulated by ERK, Nf-κB and tonEBP pathways.

TOXICOLOGÍA

541. (303) **GLYPHOSATE NEUROTOXICITY IN ADULT RATS: ANALYSIS OF NEURO-MOTOR AND BEHAVIORAL PARAMETERS**

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The neurotoxicity caused by glyphosate (Glyph) exposures has long been described in several studies; however, there are few studies that extensively evaluate an important variable such as motor activity. Dysfunctions on motor activity induced by pesticides can be determined with relatively simple techniques and may be used as a component of the FOB (Functional Observational Battery). The aim of this experiment was to determine the neuro-motor and behavioral changes caused by sub-acute administration of Glyph. Male Wistar rats 80-90 days old were treated subcutaneously with a Glyph solution (Sigma, without adjuvants) in saline phosphate buffer (PBS). One dose of 70 mg/kg was tested every 48 hours for 14 days (n=12). A group of rats was used as control (n=12) that was injected with the vehicle. Body mass was recorded daily. Two tests were carried out in control and treated animals to determine the locomotor activity: open field and elevated plus maze, according to established protocols. The test recording was done through video camera, and then the data and statistical analysis were carried out by t student method. We found that Glyph exposed rats showed a decrease in motor activity time ($p<0.05$; p=0.0194) and distance traveled ($p<0.05$; p=0.0167) by the open field test. Regarding elevate plus maze test, rats exhibited a decrease in motor activity time ($p<0.05$; p=0.0246). On the other hand, Glyph exposed animals showed a significant decrease on body weight throughout the treatment (p=0.0001). In conclusion, these findings suggest that sublethal doses of Glyph exposure alter nervous system functionality impairing motor and behavior parameters.