during the 72h exposition to 9.6 mM NaOH. In normal cells, NHE1 inhibition significantly reduced proliferation in alkaline condition (% BrdU+ cells, +NHE1= 37±1 vs -NHE1= 31±1, p<0.05 n=8). On the other hand, inhibition of NHE1 in RCC derived 786-O cells rises proliferation in media at pH 7.4. This effect is partially reverted in the presence of alkalosis (Difference in % BrdU+ cells without NHE1 pH 7.4: 14±3 vs pH 7.5: 3±1, p<0.05 n=8). In summary, the combination of alkali plus NHE1 activity reduces tumor proliferation with little effects in normal tissue. Then, this combination of treatment could be an interesting new approach to control RCC cancer.

REPRODUCCIÓN

536. (143) **AQP9 MEDIATES LACTATE TRANSPORT IN HUMAN PLACENTA AS AN ALTERNATIVE ENERGY SUBSTRATE**

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Emerging evidence shows that placental aquaporin-9 (AQP9) is not involved in the transfer of water between the mother and the fetus. However, its role in human placenta is still unknown. AQP9 is an aquaglyceroporin that also permeates other solutes such as lactate. In brain, AQP9 may transport lactate as an alternative energy substrate.
OBJECTIVE: Our aim was to evaluate the participation of AQP9 in the lactate transfer across the human placenta.
METHODS: This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were cultured in low glucose with or without L-lactate, and in presence and absence of AQP9 inhibitors (0.3 mM HgCl2, a general blocker of AQPs, or 0.5mM Phloretin, to block AQP9). Normal glucose medium was used as control. Cell viability was assessed by MTT assay and LDH release. Apoptosis indexes were analyzed by Bax/Bcl-2 protein expression ratio and TUNEL assay.
RESULTS: In low glucose medium, MTT decreased while LDH release did not change compared to controls, suggesting that cell death is not due to necrosis. Moreover, Bax/Bcl-2 ratio and apoptotic nuclei increased (n=5, p <0.02) and the blocking of AQP9 did not abrogate apoptosis. However, when explants were cultured in low glucose medium supplemented with L-lactate, explant viability and apoptotic indexes were similar to controls indicating that L-lactate could be replacing glucose as an energy substrate. In this case, the blocking of AQP9 resulted in an increase in cell death (n=4, p <0.05), proposing that this protein has a role in lactate transport.
CONCLUSION: Our results show that placental AQP9 may have a key role in lactate transport as an alternative energy substrate. Thus, the blocking of lactate transport mediated by AQP9 negatively affects the survival of trophoblast cells.

537. (232) **ASSESSMENT OF FERTILITY IN MALE MICE CHRONICALLY EXPOSED TO VARIABLE DIETARY OMEGA 3 FATTY ACID LEVELS**

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Background: dietary levels of omega-3 polyunsaturated fatty acids (ω3 PUFA) are reflected in tissue PUFA composition and can influence several processes of mammalian reproductive function. In a previous mouse study, we observed that chronic dietary ω3 PUFA deficiency produced an increase of bending immature sperm forms, which in turn can be harmful to sperm migration and oocyte fertilization. Objectives: to assess the effect of dietary ω3 PUFA level on male reproductive capacity in vivo and the oxidative stress in epididymal sperm. Material and methods: albino Swiss mice were exposed to ω3 PUFA Control (soybean oil, 7%), Deficient (sunflower oil, 7%), or Excess (blend oil; 4.2% cod-liver+2.8% soybean) diet before conception until adulthood. Five males per treatment were individually mated to two adult females fed with commercial pellet. On gestational day 18, dams were euthanized and their uteri and ovaries dissected to assess: number of live and atrophied fetuses, litter weight, number of corpora lutea and embryo loss. Other eight males per treatment were euthanized, and their epididymides dissected to evaluate the sperm production of reactive oxygen species (ROS) by chemiluminescence. Statistics: ANOVA and LSD post hoc test. Results: no significant differences in the assayed reproductive parameters were found among groups: number of fetuses ($F(2,26)= 0.63$, $P=0.53$), atrophied fetuses ($F(2,26)=0.08$, $P=0.92$), litter weight ($F(2,26)=1.17$, $P=0.32$), corpora lutea ($F(2,26)= 0.06$, $P=0.93$) and percentage of embryo loss ($F(2,26)= 1.53$, $P=0.23$). Regarding sperm ROS production, no significant differences among 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.

538. (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 signifi-