Clinical and Experimental Obstetrics & Gynecology

Conference Report

International Symposium on Reproductive Health: overcoming barriers for research in reproduction

ISRH2021 Scientific and Organizing Committee*,§

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Abstract

Accumulating evidence suggests that parental health, even before conception, may affect offspring development. Stressful environments during critical periods of growth and development that include preconception, pregnancy, and early childhood could cause long-term effects that may impact offspring's health. These environmental factors may include maternal and paternal metabolic and endocrine health, exposure to endocrine disruptors, pollutants, environmental stressors and chemicals, and also the use of assisted reproductive techniques (ARTs), among others.

Periconceptional and prenatal care are crucial to improving infants' development and health and preventing adult diseases, such as diabetes, neurocognitive, and other multifactorial and complex disorders.

Although increasing attention has been given to prenatal care management in the last years, there are still disparities among nations in terms of access to healthcare and also controversial results in many aspects, and unresolved issues. In this regard, the COVID-19 pandemic has raised new questions regarding reproduction, pregnancy and childhood development care.

In particular, in Latin America, socioeconomic inequalities in primary health system access make these societies vulnerable in terms of gestational care. Moreover, although antenatal care is more accessible in developed countries, there is still a need to comprehend the impact of different environmental cues on human health and development and improve the possible medical interventions and public policy management.

To address the above-mentioned topics, the International Symposium on Reproductive Health 2021 (ISRH2021) was proposed by a group of early-career scientists from Argentina, as a free one-day symposium with different roundtable sessions, including:

-Maternal-fetal interface

-Maternal effects on pregnancy and offspring health

-ARTs effects on embryo and offspring development

-Paternal effects on fertility and offspring health

The virtual format provided a networking space between Early-Career and experienced researchers from home, anywhere in the world. This not only allowed to join experts from Latin-American and developed countries but also

allowed a wider global audience to attend, including those who may not be able to travel for a face-to-face meeting. The economic barrier is a common problem in Latin America and developing countries as the low incomes affect the possibility of attending international meetings. Moreover, as ECRs are the academic members with lower salaries, they are usually the most affected. The spirit of this symposium was to create possibilities for worldwide participation at all career stages.

During the ISRH2021, each session consisted of two Senior talks of invited international researchers and two short talks of early-career researchers (ECRs), which were selected based on their abstract quality. Also, a poster session was held.

To generate different opportunities for interaction between Senior and ECRs, several short talks were also held, followed by a debate. Among the topics discussed were "Women in Science and Gender Discrepancy", "From basic research to public policies", "ECRs Resources" and "Career Paths".

Listed below we present the abstract of the works presented at the ISRH 2021 meeting.

WORKS SELECTED FOR ECRS ORAL PRESENTATIONS

-Maternal-fetal interface

#25 ultrastructural changes and oxidative stress in the trophoblastic cell line HTR8/SVneo induced by zika virus infection

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Zika virus (ZIKV) is an arbovirus, which can be diffused by vertical transmission causing alarming reports of Congenital Zika Syndrome cases associated with infection. Despite its importance and the studies developed, the pathogenesis of this disease has not yet been clarified. In this work, we investigated the consequences that ZIKV infection can develop on the placental trophoblastic cell lineage, HTR-8/SVneo. We standardized an infection protocol, using different virus MOIs (0.1, 0.2 or 1) per 24 h or Mock (negative control), evaluating the number of cells infected by immunofluorescence and flow cytometry. Results by immunofluorescence showed that ZIKV could infect HTR-8/SVneo, and in the infection with MOI 1 we detected a higher intensity of NS1 protein staining in relation to other MOIs, and this was the best condition, confirmed by flow cytometry, which induced the highest percentage of infected cells (44.2%). The ultrastructure of infected cells presented mitochondrial alterations, which were smaller and with loss of mitochondrial crests, endoplasmic reticulum with less dilated cisterns and the presence of many prolongations and vesicles secretion, compared to the Mock. The infected cells showed a significant increase in the activity of the antioxidant enzyme Superoxide Dismutase, and the reactive species Malondialdehyde and Nitric Oxide (1.29 ± 0.41 U/mg protein, 0.01 ± 0.001 nmol/mg protein and 95.68 ± 52.01 μmol/mL) compared to the control (0.21 ± 0.11 U/mg protein, 0.003 ± 0.002 nmol/mg protein and 48.79 ± 9.18 μmol/mL), respectively. On the other hand, the enzymatic activity of Catalase was lower in ZIKV infected cells (5.1 ± 3.8 U/mg of protein) compared to the control (33.88 ± 30.0 U/mg of protein), due to its rapid consumption and depletion in infection. These results will help to better understand the pathogenesis of the disease, as they may elucidate the consequences of infection in placental cells.

#34 Glyphosate-based herbicide alters decidual angiogenesis in neonatally exposed rats

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Glyphosate-based herbicides (GBH) are the most employed agrochemicals around the globe. Although has been considered harmless for decades, growing evidence shows that GBHs might have adverse effects on human and animal health. In previous studies, we showed that female rats neonatally exposed to GBH exhibited, at prepuberal age, altered expression of molecules involved in uterine development with higher incidence of luminal epithelial hyperplasia and increased cell proliferation. In addition, we reported a reduction of the implantation sites (IS) size and altered expression of decidualization-related molecules in association with increased post- implantation losses rates. Considering that decidualization comprises not only morphogenetic and biochemical but also vascular changes, our aim was to evaluate the effects of neonatal GBH exposure in angiogenesis on post-natal day (PND) 8 and on gestational day (GD) 9 (post-implantation period). To achieve this, Wistar female rats were s.c. exposed to saline solution (vehicle; n = 16) or to GBH (2 mg glyphosate/kg-bw/day; n = 16) every 48 h from PND1 to PND7. On PND8, eight animals from each group were euthanized, and uterine samples were collected. On PND90, the remaining animals were mated. In the morning of GD9, pregnant rats were sacrificed, and IS were collected. Histology was evaluated by H-E, while immunohistochemistry was performed to determine the VEGF, Nestin and Ki67 expression. Angiogenic-related molecules were also assessed by RT-PCR. On PND8, VEGF, Notch1, iNOS and Angpt2 mRNA levels were decreased in the GBH group. On GD9, the vascular area and vessel diameter, cell proliferation, VEGF and Nestin protein expression, and VEGF, Notch1, iNOS and COX2 gene expression were downregulated in exposed animals. In conclusion, neonatal exposure to a GBH alters the expression of molecules involved in angiogenesis during uterine development at prepubertal age and during the decidual angiogenic process. These alterations might contribute to the increased post-implantation losses observed in GBH exposed rats.

ARTS EFFECTS ON EMBRYO AND OFFSPRING DEVELOPMENT

#10065 Altered body weight of mouse offspring born after embryo transfer is preserved in the second generation

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Embryo transfer is the final step in Assisted Reproductive Technology (ART). It is known that ART procedures can lead to adverse outcomes, including lower body weight. The aim of the present study was to evaluate the impact of the embryo transfer on growth of mouse offspring and to examine whether this procedure causes changes in body weight that persist in the next generation. To obtain embryos for transfer, 3–4 months old females (CBA) were mated with males of the same age. Then, on 3.5 days post coitum (dpc), the embryos were collected by uterine flushing and transferred directly to the uterus of pseudo- pregnant females (ET group). Control group (NM) consisted of offspring developed after natural mating of females. Subsequently, males and females of the first generation (F1) were crossed with control female or male, respectively, to obtain the second generation (F2). Offspring (F1 and F2) were subjected to basic clinical screening of physical landmarks and preweaning body weight measurement. Animals born after ET were significantly smaller than animals from NM group (males: ET(13.08 ± 0.97) vs NM(16.21 ± 2.57), $p < 0.001$; females: ET(11.48 ± 0.47) vs NM(13.51 ± 2.10), $p < 0.05$). In second generation (animals born from females (ET F) and males (ET M) obtained after embryo transfer) observed similar alterations in body weight (males: ET F(12.10 ±

1.76) vs NM(16.21 ± 2.57), $p < 0.001$ and ET M(11.51 ± 2.19) vs NM(16.21 ± 2.57), $p < 0.001$; females: ET F(9.44 ± 2.43) vs NM(13.51 ± 2.10), $p < 0.001$ and ET M(11.32 ± 1.79) vs NM(13.51 ± 2.10), $p < 0.05$). Animals from ET groups in both generations did not show any difference in development of physical landmarks ($p > 0.05$) in comparison to control group. Embryo manipulation, such as embryo transfer, may cause alteration in animal growth regulation. This study shows that reduced growth trait is passed to the second generation which may be associated with epigenetic modifications related to imprinted genes.

#10066 Bioethical challenges posed by surplus frozen embryos: strategies to facilitate embryo disposition decisions in Ibero-American Countries of Catholic Cultural Tradition

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In Argentina, access to ART treatments has been regulated since 2013, but the law fails to define a number of important issues, including EDD and national registries. Disputes regarding the legal status of cryopreserved embryos are mainly associated with the influence of the Catholic Church on policy makers, and a clear resolution of embryo disposition remains a difficult topic. Also, improvements in IVF laboratory procedures, such as single embryo transfer (eSET), preimplantation genetic testing (PGT), and the freeze-all strategy, have led to an increase in the number of frozen embryos being stored. Yet, little is known of how these enhanced procedures might influence embryo disposition decisions (EDD). To collect data on storage content, an online survey was sent to all reproductive facilities, during 2017 and 2020. Based on the survey results, we found a tendency that shows an exponential increase in the number of frozen embryos (by 68.5%). This is a consequence of the improvements in cryopreservation techniques (vitrification) and the development of more efficient ovarian stimulation protocols that have facilitated a rise in elective single embryo transfer (eSET). This paper focuses on three strategies that could be implemented to facilitate EDD under this particular setting. First, counseling sessions at different treatment stages should be encouraged and would be conducted by trained mental health professionals. Second, once storage content is labeled, embryos which were cryopreserved more than 10 years ago and aneuploid embryos, could form part of a national bank for research purposes. Third, promote effective regulation that includes EDD and explicit storage limits. This research is part of the project Social Challenges of Medically Assisted Human Reproduction in Ibero-American Countries of Catholic Cultural Tradition funded by the Ibero-American Union of Universities (UIU).

-Paternal effects on fertility and offspring health

#60 Seminiferous tubule length and sperm production in low birth weight sexually matured male pigs

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Given the high incidence of low birth weight piglets and the importance of males as semen donors for artificial insemination, the aim of this work was to evaluate testicular morphological parameters associated to semen characteristics in different birthweight sexually matured boars. For this, twenty-four newborn littermate boars were selected after birth and divided into two experimental groups (n = 12/group), according to birth weight. High (HW), ranging from 1.80 to 2.25 kg and Low (LW), ranging from 0.75 to 1.10 kg. Both groups were submitted to biometrical analyses; Bodyweight was measured at birth and at 300 days of age and testis weight and volume at 300 days. At 170 days of age, a sub-set of 14 littermate boars (n = 7 HW and n = 17 LW) were randomly selected and semen samples were collected every two weeks for further evaluation. At 300 days of age, all males were orchiectomized and the testis were fixed, by immersion, in paraformaldehyde for histomorphometrical analyzes. HW males presented greater body weight and testicular measures compared to LW animals ($P < 0.05$), but birthweight did not affect semen quality parameters (volume, concentration, motility, vigor and morphology). Concerning histological parameters, the seminiferous tubule diameter and epithelium height were not affected by birthweight, but LW animals showed shorter seminiferous tubule length ($P < 0.05$). In addition, LW mature boars showed a 45% drop in sperm production. Regarding semen morphology, both LW and HW boars showed no evidence of spermatogenesis defects or semen pathologies. Furthermore, birthweight was positively correlated to total seminiferous tubule length (r = 0.75 and $P < 0.01$) and total seminiferous tubule length was positively correlated to body (r = 0.63) and testis (r = 0.89) weights ($P < 0.05$). Thus, despite the good quality of LW's semen, mature HW boars showed potential to produce more spermatozoa and more semen doses per ejaculated and would be highly valuable for the artificial insemination industry.

#44 Sperm selection by thermotaxis in bull and stallion

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Sperm thermotaxis is the ability of the spermatozoa to move in a temperature gradient towards the warmer temperature. It seems to be exclusive of capacitated spermatozoa and appears to be conserved within different mammal species. Our objective was to assess sperm selection by thermotaxis in both stallion and bull frozen semen. After sperm migration by thermotaxis, we evaluated the number of cells migrated for both species. In stallion, we also analysed the DNA fragmentation index with COMET Assay and the protein tyrosine phosphorylation (PTP) with immunocytochemistry whereas in bull we analysed the acrosomal exocytosis. Net thermotaxis was assessed by subtracting the number of spermatozoa migrated by thermotaxis by the number of spermatozoa migrated in the control. Using ejaculates from 5 stallions (n = 5) and 4 bulls (n = 4), we reported a net thermotaxis of 1.1% ± 0.5% for stallion and 0.78% ± 0.50% for bull. In bull, acrosomal exocytosis was similar in the spermatozoa selected by thermotaxis and the control at 37 ºC (2.3% ± 4.5% and 3.7% ± 2.9%, ONE way ANOVA). In stallion, similar levels of PTP were found in the flagellum of the selected spermatozoa compared to the 38 ºC control (37% ± 8% and 24% ± 8%, ONE way ANOVA). However, these levels were significantly higher when compared to the sample kept at 37 ºC ($p < 0.02$). Regarding DNA fragmentation, lower levels were found in the migrated sperm vs. sperm in the control at 37 ºC (4.4% ± 0.4% and 11% ± 2%, $p = 0.009$, Student's *t*-test). We are now evaluating the quality of the selected bovine spermatozoa using fertilization by ICSI. This is the first study revealing thermotactic behaviour of bull and stallion spermatozoa, which is similar to that observed in mice and human, suggesting commonality of thermotaxis mechanisms in mammalian spermatozoa.

-Maternal effects on pregnancy and offspring health

#33 A diet enriched in extra virgin olive oil prevents placental morphologic alterations and maternal serum matrix metalloproteinases overactivity in GDM patients

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Gestational diabetes mellitus (GDM) is a prevalent disease that increases the risks of maternal, fetal and placental complications and leads to the programming of metabolic diseases. Changes in placental morphology are associated to an adverse programming. Matrix metalloproteinases (MMPs) are proteolytic enzymes, markers of a pro-inflammatory state if overexpressed. Our studies have shown that a dietary supplementation with extra virgin olive oil (EVOO) is capable of preventing increased maternal weight gain, circulating triglycerides and placental MMPs. In this work, we hypothesized that a maternal diet enriched in EVOO modulates placental morphology, circulating cholesterol levels and maternal blood MMPs activity in women with GDM.

Methods: Fifty healthy (Control) and patients with GDM were enrolled after signing an informed consent (protocol approved by the Ethics Committee of Hospital Pirovano, Buenos Aires). All of them were advised to follow the WHO diet for pregnancy. Patients with GDM were randomized to receive or not three tablespoons/day of EVOO from week 24–28 of gestation until delivery. Placental samples and maternal blood were obtained at term. MMPs activity was evaluated by zymography.

Results: Although placental diameter and perimeter were similar in the three evaluated groups, the placentas from the GDM group were thicker compared to controls (15%, $p < 0.05$), an alteration prevented by the EVOO-enriched diet ($p < 0.05$, GDM-EVOO vs. GDM). Total cholesterol and HDL-cholesterol in maternal blood showed no changes. Serum MMP-9 activity was increased in the GDM group (81%, $p < 0.01$ vs. Control), an alteration prevented by the EVOO-enriched diet ($p < 0.05$ vs. GDM).

Conclusion: Maternal GDM led to increased placental thickness, an alteration that may impair placental transport function and was prevented by the EVOO-enriched diet. The capacity of EVOO treatment to prevent maternal blood MMPs overactivity evidences its beneficial effects in the mother, and identifies MMP activity as a putative biomarker of the pro-oxidative status.

#24 Machine learning reveals maternal thyroid variables as a potential tool to improve the diagnosis of gestational diabetes mellitus

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Brief Introduction: Gestational diabetes mellitus (GDM) is a hyperglycemia state that is diagnosed during the second or third trimester of pregnancy, typically by an oral glucose tolerance test (OGTT). Finding ways to improve its diagnosis could help to optimize preventive interventions and avoid its negative consequences on maternal and fetal health, short and long-term.

Objective: To identify maternal gyneco-obstetric variables that may improve the diagnosis of GDM in Chilean pregnant women.

Methods: Pregnant women with ≤12 gestational weeks (GW) and without pregestational diabetes were recruited in Concepcion, Chile. During the first (1T) and the second (2T) trimester of pregnancy, 29 gyneco-obstetric variables were registered. GDM diagnosis was performed at 24–28 GW, with postload glycemia (75 g, 2 h) ≥140 mg/dL. Out of 39 women, 6 had GDM. Data were preprocessed by autoscale and explored by the machine learning technique Principal Component (PC) Analysis.

Main Outcomes and Results: When data from both trimesters are used, GDM women can be distinguished from the non-GDM ones by PC2 (14% variance). This separation is mainly attributable to the following variables: OGTT, prior GDM, total T3 (2T), TSH (2T), free T4 (2T), total T4 (2T), diastolic pressure and total T4 (1T). Interestingly, when removing OGTT, the separation between the two groups (PC2, 12% variance) is maintained, and referable to: free T4 (2T), total T4 (2T), total T4 (1T), diastolic pressure, prior GDM, total T3 (2T) and TSH (2T). Moreover, when using only data from 1T, the GDM group can still be distinguished from the non-GDM one. In this case, the separation (PC6, 7% variance) is attributable to: FTO genotype, prior GDM, total T3 (1T), diastolic pressure, anti-TR and anti-TPO.

Conclusions: Maternal thyroid variables are relevant to discriminate GDM from non-GDM women in both 1T and 2T. Therefore, they may be helpful to improve GDM diagnosis.

ABSTRACTS SELECTED FOR ECRS POSTER PRESENTATIONS

-Maternal-fetal interface

#16 Aquaporin-1 and Caveolin-1 are essential for the proper formation of the placental vasculature

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Introduction: Placental angiogenesis requires proliferation, migration, and differentiation of endothelial cells. There are large numbers of caveolae in the plasma membrane of endothelial cells. Caveolae are lipid domains involved in vesicular trafficking and signal transduction. Caveolin-1 (Cav-1) is the main protein that forms caveolae, and can bind to different membrane proteins such as aquaporins. Aquaporin-1 (AQP1) is a transmembrane water channel that moves water in response to osmotic gradients. Regarding placental macrovascular endothelial cells, we previously demonstrated that AQP1 and Cav-1 are expressed in these cells.

Objective: To evaluate the role of AQP1 and caveolae/Cav-1 in the formation of the placental vasculature.

Materials and Methods: Macrovasculature cell line EA.hy926 (ATCC® CRL-2922™) was used. Co-localization of Cav-1 with AQP1 was assessed by confocal immunofluorescence. Putative Caveolin-1 binding sites on the amino acid sequence of AQP1 were analyzed by *in silico* analysis using BioEdit and PyMol programs. Cells were treated with 5 mM methyl-ß-cyclodextrin (MßCD) to disrupt caveolae, and with tetraethylammonium chloride (TEA) 50 μM and 100 μM to block AQP1. The efficiency in the blockade of AQPs was evaluated by cell swelling assay. Cell viability was assessed by MTT and cell migration by wound healing assay.

Results: *In silico* analysis showed that AQP1 has a putative binding site for Cav-1. In addition, Cav-1 and AQP1 co-localize in the cell membrane of EA.hy926 cells. Treatment with MßCD to disassembly the caveolar structure

significantly reduced cell migration ($p < 0.05$, n = 4). The blocking of AQP1 with TEA also reduced endothelial cell migration ($p < 0.001$, n = 4).

Conclusions: Our results suggest that intact caveolar structures, as well as the presence of a functional AQP1, are necessary for the correct migration of placental endothelial cells. Any perturbations might result in aberrant angiogenesis leading to serious pregnancy disorders such as preeclampsia or fetal growth restriction.

#19 High levels of tumor necrosis factor-alpha reduces placental aquaporin 3 expression and impairs *in vitro* trophoblastic cell migration

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Introduction: Placentas from preeclamptic women display augmented tumor necrosis factor-alpha (TNF-a) levels with reduced expression of aquaporin 3 (AQP3). However, whether TNF-a modulates AQP3 expression remains to be elucidated.

Objective: To characterize if elevated levels of TNF-a reduces AQP3 expression and negatively impacts on trophoblastic cell migration.

Methods: Spontaneously hypertensive rats (SHR) and Wistar (14–16 wk) were divided into hypertensive (n = 6) and normotensive (n = 6) groups. Systolic blood pressure (SBP) was measured, and animals mated. In a third group, pregnant SHRs (n = 8) were treated with a TNF-a antagonist, etanercept (0.8 mg/kg, i.p.) on days 0, 6, 12, and 18 of pregnancy. Placentas were collected on the 20th day of pregnancy. Human placental explants (n = 7), from normotensive pregnancies, were incubated with TNF-a (5, 10 and 20 ng/mL) and/or etanercept (1 μg/mL). Swan 71 cells were incubated with TNF-a (10 ng/mL) and/or etanercept (1 μg/mL) and subjected to the wound healing assay. AQP3 expression was assessed by western blot and TNF-a levels by ELISA.

Results: SBP (mmHg) was elevated in the hypertensive group, and etanercept treatment reduced this parameter. Placental TNF-a levels (pg/mL) were higher in the hypertensive group. AQP3 expression was reduced in the hypertensive group, and etanercept treatment reversed this parameter. Explants submitted to TNF-a exposition displayed reduced expression of AQP3 and etanercept incubation reversed it. Trophoblastic cells incubated with TNF-a showed decreased cell migration and reduced AQP3 expression and etanercept incubation ameliorated it.

Conclusion: Altogether, these data demonstrate that high TNF-a levels negatively modulates AQP3 in placental tissue, impairing cell migration, and its relationship in a pregnancy affected by hypertension.

#23 Maternal and fetal thyroid hormones are associated with altered deiodinase expression and activity in placenta with gestational diabetes

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Gestational Diabetes (GD) is characterized by abnormal maternal D-glucose metabolism. Dysregulation of thyroid hormones (TH) tri-iodethyronine (T3) and L-thyroxine (T4) Hormones had been associated with GD, but the physiopathological meaning of these alterations is still unclear. Maternal TH cross the placenta through TH Transporters and their Deiodinases metabolize them to regulate fetal TH levels. Currently, the metabolism of TH in placentas with GD is unknown, and there are no other studies that evaluate the fetal TH from pregnancies with GD. Therefore, we evaluated the levels of maternal TH during pregnancy, and fetal TH at delivery, and the expression and activity of placental deiodinases from GD pregnancies. Pregnant women were followed through pregnancy until delivery. We collected blood samples during 10–14, 24–28, and 36–40 weeks of gestation for measure Thyroid-stimulating hormone (TSH), Free T4 (FT4), Total T4 (TT4), and Total T3 (TT3) concentrations from Normal and GD mothers. Moreover, we measure fetal TSH, FT4, TT4, and TT3 in total blood cord at the delivery. Also, we measured the placental expression of Deiodinases by RT-PCR, western-blotting, and immunohistochemistry. The activity of Deiodinases was estimated quantified rT3 and T3 using T4 as a substrate. Mothers with GD showed higher levels of TT3 during all pregnancy, and an increase in TSH during second and third trimester, while lower concentrations of neonatal TT4, FT4, and TT3; and an increased TSH level in umbilical cord blood from GD. Placentae from GD mothers have a higher expression and activity of Deiodinase 3, but lower Deiodinase 2, than normal mothers. In conclusion, GD favors high levels of TT3 during all gestation in the mother, low levels in TT4, FT4 and TT3 at the delivery in neonates, and increases deiodinase 3, but reduce deiodinase 2 expression and activity in the placenta.

#29 VIP-deficient mice pregnancy exhibit growth impairment, increased placental glucose uptake and altered transplacental transport

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Background: Glucose uptake by the placenta and transplacental transport are finely regulated processes required for placental and fetal development. Deficient placentation is associated with pregnancy complications such as preeclampsia and fetal growth restriction as well as fetal programming for diseases in adult life. The vasoactive intestinal peptide (VIP) has embryotrophic effects in mice and regulates human cytotrophoblast function and metabolism.

Objective: Our aim was to evaluate the in vivo placental glucose uptake and transfer to the fetus in WT or VIP-deficient placentas and we investigated the role of endogenous VIP in the regulation of placental glucose and amino acid uptake.

Design: Wild type C57BL/6 (WT) females were mated with WT or VIP knock-out (VIP KO) males. Glucose uptake and transplacental transport were evaluated by the injection of 3 mM D-glucose analogue 2-NBDG in pregnant mice at gestational day 17.5. Placental gene expression was measured by RT-qPCR. For ex vivo experiments, VIP+/+ placental explants were incubated with 100 nM VIP antagonist prior to the addition of 2-NBDG or 14C-MeAIB to evaluate glucose and amino acid uptake, respectively.

Results: Placental weight was unaltered due to placental VIP deficiency, whereas embryonic growth was impaired. Paradoxically, VIP+/- placentas presented with higher glucose uptake and GLUT1/mTOR gene expression compared with VIP+/+ placentas ($p < 0.05$) while a trend to decrease of placental IGFII, LeptinR and insulin expression was observed. VIP antagonist application to WT placental explants independently impaired glucose and amino acid uptake ($p < 0.05$).

Conclusions: Our results point to a regulatory role of VIP in modulating placental metabolism and glucose transplacental transport in vivo. Endogenous VIP sustains placental glucose and amino acid uptake whereas VIP deficiency triggers compensatory pathways that would contribute to placental metabolic adaptations. The apparently compensatory actions are not sufficient to sustain normal fetal growth and could result in complications later in life.

#35 Anandamide and cyclooxygenase-2 participate in vascular remodeling at the maternal-fetal interface

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Successful implantation and placentation requires vascular transformation of the uterus. A dynamic interaction between cells at the maternal-fetal interface is crucial for these processes. Anandamide (AEA) is an endocannabinoid that regulates embryo implantation and many of the placental functions. The uterine levels of AEA are primarily regulated by fatty acid amide hydrolase (FAAH), its degrading enzyme. Using *in-vivo* and *in-vitro* approaches we investigated the role of anandamide in the vascular remodeling of the uterus at early gestation. Also, ciclooxigenase-2 (COX-2) participation was studied. First, Wistar rats received an intrauterine injection of URB-597 in day 5 of gestation (day of implantation). URB-597 is a highly selective inhibitor of FAAH. Control horns were injected with vehicle. Animals were euthanized in day 8. We observed that: (1) URB-597 increased fetal resorptions and induced aberrant embryo spacing and abortions, (2) URB-597 augmented the cross-sectional length of the uterine and arcuate arteries, and (3) Meloxicam (a highly selective COX-2 inhibitor) prevented URB-597 effects. Afterward, we studied the effect of AEA in the interaction between the endometrial stromal fibroblasts and the endothelial cells of the maternal vessels. Thus, human endometrial stromal cells (T-hESC) were incubated with AEA or AEA + meloxicam. AEA stimulated T-hESC migration in a concentration-dependent manner and COX-2 mediated this effect. To test endometrial fibroblasts-endothelial crosstalk, the endothelial cell line EA.hy926 was incubated with T-hESC conditioned medium. The conditioned media from AEA-induced T-hESC migration stimulated endothelial cells migration. Soluble factors derived from COX-2 pathway were involved in T-hESC and EA.hy926 interaction. Collectively, our results show the participation of AEA in the vascular remodeling that takes place in the uterus during early gestation by a mechanism that involves the COX-2 isoform.

#39 Detection of Shiga toxin producing Escherichia coli in endocervix of asymptomatic pregnant women: novel pathogen responsible for adverse pregnancy outcomes?

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Brief Introduction: Some studies have demonstrated that vaginal E. coli colonization may produce complications during pregnancy. We have previously reported that Shiga toxin-2 (Stx2) secreted by enterohemorrhagic E. coli can produce abortion and premature delivery in animals and can impair human trophoblast *in vitro*.

Objective: The aim of this study was to detect virulence factor genes from Shiga toxin producing E. coli (STEC) in the endocervix of asymptomatic pregnant women. Methods: Endocervical swabs were collected from 103 pregnant

women (12 to 30 weeks of pregnancy) during their antenatal examination. Swab samples were enriched in Tryptic Soy Broth overnight at 37 °C and then streaked into selective and differential medium, Sorbitol MacConkey agar. E. coli detection was confirmed by identification of uidA gene by PCR assay. Then, positive samples for E. coli were analyzed for STEC virulence factors genes: stx1, stx2, eae, rfbO157, lpfAO113 and hcpA. After that, positive E. coli samples for stx2 gene were grown in Luria-Bertani Broth medium in order to evaluate Stx2 cytotoxic activity. For that, the bacterial supernatants were filter-sterilized and cytotoxicity was evaluated on Vero, Swan 71 and HeLa cells.

Results: Our results showed that 14.5% of the endocervical samples were positive for E. coli (positive for uidA gene). Furthermore, 9/15 (60%) of the E. coli-positive samples carried the stx2 gene and 6/15 (40%) carried the lpfAO113 and hcpA genes. One bacterial supernatant from an E. coli-positive endocervical sample carrying stx2 and lpfAO113 genes exhibited high cytotoxic activity on the cells evaluated due to Stx2 production confirmed by specific neutralization of Stx2 with an anti-Stx2 antibody.

Conclusions: The novelty of this report is the presence of STEC in the endocervix of asymptomatic pregnant women. This opens a new perspective with respect to the possible role of this pathogen in woman reproductive health care.

#42 AQP9-mediated lactate transport: an alternative source of energy in placenta

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Introduction: Emerging evidence shows that placental AQP9 is not involved in the transfer of water between the mother and the fetus. AQP9 is an aquaglyceroporin that also permeates other solutes such as lactate. In other tissues, AQP9 may transport lactate as an alternative energy substrate, having important participation as a nutrient sensor, detecting the availability of nutrients in the cell.

Objective: Our aim was to evaluate the participation of AQP9 in the lactate transfer across the human placenta.

Methodology: 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 0.5 mM Phloretin, to block AQP9). Medium supplemented with glucose 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 ($n = 4$, $p < 0.05$).

Conclusions: Our results show that placental AQP9 may have a key role in lactate transport as an alternative energy substrate on nutritional stress conditions. Thus, the blocking of lactate transport mediated by AQP9 negatively affects the survival of trophoblast cells in these conditions.

#45 Endocannabinoid system from maternal-fetal interface is involved in preterm birth induced by LPS

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Preterm birth (PTB) is the leading cause of mortality and morbidity in neonates. It is well known that premature deliveries are mainly associated with infectious processes and the stability of the maternal-fetal interface is essential for the maintenance of pregnancy. The endocannabinoid system (ECs) is one of several signaling pathways involved in different aspects of the physiopathology of reproduction. The present study aimed to investigate the participation of ECs in the maternal-fetal interface, using an LPS-induced preterm labor model.

Our group developed a murine model of preterm labor, induced by two injections of LPS on day 15 of pregnancy, that produces an 85% of PTB in Balb/C mice.

Using this model, we observed in deciduas from LPS treated mice a decrease in CB1 receptor protein level vs control deciduas ($p < 0.05$). Conversely, CB1 receptor protein levels increased in PBMC after LPS treatment.

Regarding the enzyme that synthesizes AEA, (N-acylphosphatidylethanolamine-specific phospholipase D, NAPE-PLD), we observed that its protein levels were diminished in both, deciduas and PBMC, from LPS treated mice.

On the other hand, the enzyme that degrades AEA (fatty acid amide hydrolase, FAAH) protein levels and activity were not modified by LPS treatment in deciduas. However, FAAH protein levels and activity were diminished in PBMC after LPS treatment ($p < 0.05$).

To analyze if the CB1 receptor is involved in LPS-induced preterm birth, we used a CB1-KO mice. We injected two different doses of LPS (10 ug/mice and 20 ug/mice of weight) and we observed that CB1-KO mice presented a lower preterm birth rate than WT mice.

In summary, these data suggest that endocannabinoids are involved in pro-inflammatory response associated with LPS-induced preterm birth and that CB1 receptor is implicated in the triggering of preterm birth.

#49 Impaired development blastocysts induce an inflammatory response on decidualized cells

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Introduction: human decidualization program starts on each menstrual cycle during the secretory phase. Decidualized cells are receptive to embryo implantation but also selective to their quality, so impaired/low quality embryos invasion is restricted. Here we hypothesize that impaired development embryos induce an exacerbated inflammatory response that avoids implantation and triggers menstruation.

Methods: Human endometrial stromal cell line (HESC) was decidualized with medroxyprogesterone + dbcAMP during 8 days. Then, decidualized HESC cells were stimulated with human blastocyst conditioned media (BCM) from day 5 developing (normal development: ND) or arrested (impaired development: ID) blastocysts. Blastocysts were obtained from IVF/ICSI and classified according to morphological criteria. Non decidualized cells were used as control. Cytokine expression/production was evaluated by RT-qPCR/flow cytometry/ELISA. Neutrophils and Tregs were obtained from peripheral blood from healthy donors, and their migration towards HESC supernatants was evaluated using a transwell system. Neutrophils ´ROS production was assessed by CDFH-DA probe.

Main outcomes and results: ID-BCM stimulation increased IL-1ß production compared to decidualized cells (Dec), while ND-BCM reduced IL-1ß production. When a cytokine profile was evaluated, we observed that ID-BCM increased CXCL12 expression as well as IL-8 secretion. Since IL-8 and CXCL12 are associated to neutrophils recruitment towards the inflammatory sites, we evaluated neutrophils migration using a transwells system. In this sense, ID-BCM increased neutrophils recruitment. Additionally, when neutrophils were stimulated with HESC supernatants, ID-BCM treatment leaded to higher ROS production, suggesting an increased activation of these immune cells. On the contrary, ID-BCM showed a reduced Tregs recruitment compared to Dec treatment.

Conclusions: Decidualized cells respond to impaired development blastocyst derived factors inducing a pro-inflamatory microenvironment. These findings provide new clues about the embryo maternal cross-talk and the natural embryo selection; and might contribute to a better understanding of reproductive disorders such as *in vitro* implantation failures.

#50 Age of first sexual intercourse and menarche in adolescents with mental disorders

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Introduction: Adolescents with mental disorders are more sexually vulnerable according to several studies that identify greater risks of sexual violence and unintended pregnancy in this population.

Objective: Identify the age at which the first menstruation and the first sexual intercourse occur in Adolescents with Mental Disorders.

Method: The study was carried out at ADOLESCENTRO, a reference center for the care of adolescents with mental disorders, in the Federal District/Brazil. During a 12-month period, 1232 adolescents aged 10 to 19 years were attended to, where 862 were sexually active and wanted safe contraception. Of this group, 96 agreed to participate in this study, which involved the use of contraceptives, and several variables were evaluated. The group was considered to be at high risk for unintended pregnancy, as all had mild and moderate mental disorders, such as depression, anxiety, bipolar disorder, ADHD, intellectual disability and schizophrenia. During the study, all were undergoing treatment, 85% of whom used psychiatric medication. They always attended accompanied by their tutors.

Results: Regarding the age of menarche, of the 96 adolescents, 29 (30%) menstruated at 12 years old, while 24 (25%) at 13 years old. When we add 11 to 13 years old, we have 65 (68%) of adolescents with the beginning of cycles. At earlier ages, menarche occurred at 9 years of age in 8 (8.3%) and at 10 years of age in 10 (10.3%) of them. At the age of 14, 9 (9.4%) and at the age of 15, only 2 (2%) menstruated. Only 2 (2%) ignored this data. Considering the age of sexual initiation, including cases of declared and legally presumed sexual violence (below 14 years old), we find the following data: the predominant age of the first sexual intercourse was at 14 years old in 31 (32.3%) of the girls. Between 13 and 15 years of age, 68 (70%) of them had started their sexual activities. At 12 years of age, 7 (7.3%) of them had their first intercourse. Between 16 and 18 years old, 16 (16.7%) of them had their first sexual intercourse. And finally, at the extremes, at 9 years old 1 (1%), 11 years old 2 (2%) and at 18 years old 1 (1%) reported having started their sexual activity. Two adolescents (2%) ignored this data.

Conclusion: Considering the great risk of pregnancy, sexual violence, the difficulty in self-care and access to contraceptive methods, the results showed that there is an immense need to promote and guarantee the sexual and reproductive rights of this invisible and often excluded population from planning actions reproductive.

#53 Neutrophil-trophoblast cell interaction in early pregnancy: regulatory mechanisms and immunometabolism

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Maternal leukocytes are recruited to the maternal-placental interface from the post implantatory period until delivery and both normal placentation and the success of pregnancy strongly depend on an appropriate communication established with trophoblast cells. Defects in these processes are associated with pregnancy complications like preeclampsia, where a neutrophil over activation is observed. In line with this, many metabolic

pathways have been shown to affect the functional role of immune cells in a number of settings but insights into immunometabolic reprogramming in the context of reproduction are still enigmatic.

For these reasons, the aim of this work was to evaluate the effect of trophoblast derived factors in neutrophils' functional and metabolic profile.

Methods: Supernatants from human first trimester trophoblast cell line Swan-71 (conditioned media, CM) were cultured with peripheral blood neutrophils from healthy donors to assess immune functional profiles and metabolism. Neutrophil profiles were assessed by RT-qPCR and flow cytometry. Treg induction was explored culturing neutrophils with autologous mononuclear cells and CD4, FOXP3 staining, followed by flow cytometry. Glucose uptake was assessed using the fluorescent analog 2-NBDG and analysis of intracellular lipid accumulation was performed using BODIPY, both by flow cytometry.

Results: Factors released by trophoblast cells shaped neutrophils to a proangiogenic profile with increased expression of VEGF, Arginase-1, TGF-ß and CCL2 ($P < 0.05$, n = 6). In the Treg induction experiment, neutrophils that were pre-incubated with CM increased the frequency of CD4 + FOXP3 + cells (% ± SE Basal: 3.85 ± 0.64, CM: 9.97 ± 3.38, $P < 0.05$, n = 6).

Regarding neutrophils' metabolism, we found that neutrophils incubated with CM of trophoblast cells, presented an increase in glucose uptake (MFI ± SE Basal: 897.3 ± 195.4; CM: 1112 ± 244.8, $P < 0.05$, n = 10) and in lipid droplet accumulation (MFI ± SE Basal: 997.7 ± 93.41 CM: 1318.0 ± 115.5, $P < 0.05$, n = 8).

Conclusions: Our results support a novel immunomodulatory role of trophoblast factors on neutrophil metabolism, providing new clues for pharmacological targeting of immune and trophoblast cells in pregnancy complications associated with exacerbated inflammation.

#55 Endoplasmic Reticulum Stress through ATF6a pathway induces an inflammatory response: potential regulation by miRNAs

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Introduction: During decidualization, endometrial stromal cells undergo endoplasmic reticulum stress (ERS) and unfolded protein response (UPR), which will allow them to expand their endoplasmic reticulum with the corresponding machinery for protein folding. These processes are directed by miRNAs that regulate the expression or stability of their transcription factors.

Objective: we focus on the role of ERS/UPR during decidualization to induce a physiological sterile inflammatory response and whether it might be regulated by miRNAs.

Methods: We used an *in vitro* model of decidualization represented by human telomerase-immortalized endometrial stromal cell line St-T1b; and endometrial biopsies from patients with recurrent spontaneous abortions (RSA) and recurrent in vitro fertilization failures (RIF).

Main outcomes and results: We evaluated the expression of the ERS-sensor ATF6 and the UPR marker, CHOP. Both markers increased in decidualized cells, and Tg (ERS inducer) induced even higher levels in comparison with non-decidualized cells ($p < 0.05$, *t*-test). Then, we evaluated the modulation of TXNIP, a link between the ERS-pathway and inflammation. TXNIP increased in decidualized cells, and also the inflammasome NLRP3 and IL-1ß expression ($p < 0.05$, *t*-test). Then, using an *in silico* analysis using miRTarBase v8.0 we selected two miRNAs able to regulate the ERS and UPR pathways: miR-193b-3p and miR-21-5p. Both miRNAs significantly decreased in non-decidualized cells in the presence of Tg ($p < 0.05$, *t*-test). Finally, we studied the expression and localization of miRNAs

through an *in situ* hybridization (ISH) technique in endometrial samples. Even both miRNAs were expressed in stromal and epithelial glandular cells in endometrial samples from RSA and RIF patients; endometrial samples from RSA patients displayed lower expression in comparison with those from RIF patients.

Conclusions: The present results suggest that decidualization in St-T1b cells is accompanied by an ERS and UPR associated with a sterile inflammatory response potentially regulated by miR-193b-3p and miR-21-5p.

#61 LPS from Porphyromonas gingivalis impairs trophoblast function and trophoblast-neutrophil interaction

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Introduction: Porphyromonas gingivalis (Pg) is an important pathogen of periodontal disease, has been implicated in adverse pregnancy outcome although the mechanisms involved are still unclear. Lipopolysaccharide from Porphyromonas gingivalis (Pg-LPS) is the main virulence factor of Pg. During placentation, trophoblast cells secrete cytokines and chemokines in order to interact with immune cells regulating and maintaining immune homeostasis. In fact, neutrophil activation is associated with poor placentation and severe pregnancy complications.

Objectives: The aim of this study was to examine the effect of Pg-LPS on trophoblast cell function and to explore TLR4/TLR2 mediated mechanisms.

Methods: Swan-71 human trophoblastic cell line was treated with Pg-LPS. Cytokine and chemokine expression were evaluated by RTqPCR or flow cytometry, glucose uptake by flow cytometry using the fluorescent analogue 2-NBDG and cell invasion assessed in Matrigel-covered transwells. Peripheral blood neutrophils were purified from healthy donors and cultured with conditioned media of trophoblast cells (TbCM) treated or not with LPS (PgLPS-CM); apoptosis was determined by fluorescence microscopy and CD11b and reactive oxygen species (ROS) were evaluated by flow cytometry.

Results: Pg-LPS treatment reduced cell migration and invasion (N = 4 $P = 0.05$). We also found that Pg-LPS treatment reduced glucose uptake ($P = 0.05$) and decreased GLUT-1 expression. Pg-LPS treatment also impaired the balance of cytokines and chemokines. Depending on the function studied TLR2 and/or TLR4 signalling pathways appeared differentially involved. Conditioned media from trophoblast cells with Pg-LPS increased neutrophil activation with higher release of ROS and lower apoptosis rate.

Conclusions: In summary, our results indicate that P. gingivalis lipopolysaccharide activation of TLR2 and TLR4 on trophoblast cells affects trophoblast cell invasion, cytokine expression and glucose uptake. leading to a deficient regulation of neutrophil proinflammatory profile This mechanism underlying Pg infection during placentation might contribute to the pathogenic effect of this bacteria on pregnancy outcome.

#62 Evaluating the contribution of X chromosome inactivation to sex dimorphic neural tube defects in SR-B1 deficient mice

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Neural tube defects (NTD) are congenital malformations caused by abnormal formation of the brain and spine. About 2 : 3 NTD cases are female, which has been related to defective female X-chromosome inactivation (XCI). SR-B1, the main HDL receptor, participates in bidirectional lipoproteins-to-cells lipid transport and is expressed in early extra-embryonic tissues. SR-B1-/- embryos are vitamin E deficient and develop NTD with a 1 : 2 prevalence and a 70 : 30% female:male proportion. Here, we evaluated if abnormal XCI may explain the female skew in NTD in SR-B1-/- embryos. We hypothesized that female embryos with NTD exhibit reduced expression of XCI regulatory genes and abnormal X-chromosome gene dosage. Massive mRNAseq data from female embryos at E9.5 [SR-B1+/+, morphologically normal SR-B1-/- (nSR-B1-/-) and NTD-SR-B1-/-; n = 3 pools of 3 embryos each] was analyzed using R Software and GSEA and GEO Analysis. Among 24,421 analyzed genes, differential expression vs. SR-B1+/+ was found in 62 and 1159 genes in nSRB1-/- and NTD-SR-B1-/-, respectively ($p < 0.05$, Wald test). Of 22 genes listed as involved in XCI, only Pcgf5 was upregulated in NTD vs. SR-B1+/+ and nSR-B1-/- embryos ($p < 0.01$, Wald test), while Xist and Tsix just tended to be higher. Global gene expression in the X-chromosome, and not in autosomes, was consistently higher in NTD-SR-B1-/- vs. nSR-B1-/- and SR-B1+/+, and in nSR-B1-/- vs. SR-B1+/+ embryos ($p < 0.01$, GSEA analyses). Interestingly, the proportion of upregulated vs. downregulated genes was higher in the X-chromosome than in autosomes in NTD-SR-B1-/- vs. the other groups ($p < 0.01$, Fisher's test). According to GOslims, the overexpressed X-linked genes in NTD-SR-B1-/- embryos are mainly involved in biological processes such as signal transduction, anatomical structure development, cell differentiation and response to stress. Together, these results suggest that an abnormally high X-chromosome gene expression, potentially due to defective XCI, may affect relevant developmental pathways and contribute to the female prevalence in NTD-SR-B1-/- embryos.

#70 Maternal vitamin E supplementation prevents neural tube defects increased by maternal high fat-high sugar intake

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Scavenger Receptor SR-B1 participates in vitamin E (VE) transport between lipoproteins and cells and is expressed in parietal yolk sac (pYS) during early pregnancy. SR-B1 KO embryos from heterozygous (HET) intercrosses exhibit neural tube defects (NTD), VE deficiency and high levels of reactive oxygen species, all of which are prevented by maternal VE-supplementation. In this work, HET dams were fed a pro-oxidative high fat/high sugar (HF/HS) diet alone or supplemented with 2000 UI of alpha-tocopherol (aT) for 2 months before mating with HET males, and NTD incidence and VE levels were evaluated in maternal plasma, pYS and embryos at E9.5. HF/HS-fed dams showed impaired i.p. glucose tolerance (area under curve [AUC]: 14537 vs. 7305 AU in chow-fed, n = 11–19, $*p < 0.05$, One-Way ANOVA + Dunn's post Test), which was prevented by VE supplementation (AUC: 9618, n = 10). NTD incidence increased with both SR-B1 deficiency and maternal HF/HS exposure ($*p = 0.017$, Spearman Rank test), and was prevented by maternal VE supplementation (KO: $**p = 0.056$, HET = ***0.0007, Chi-square test, n = 16–31 females/group). Levels of aT were similar in HF/HS vs. chow maternal plasma and embryos, but lower in pYS of all genotypes from HF/HS v chow dams (WT: 0.40 vs. 0.14, HET: 0.2 vs. 0.14, KO: 0.33 vs. 0.12 ng aT/μg protein, n = 5–9 embryos/group), even in the VE supplemented group (WT: 0.14, HET: 0.13, KO: 0.06, 6–7 embryos/group, $***p = 0.0005$, Two-Way ANOVA + Bonferroni). Our preliminary data in HET pYSs show normal levels of phosphorylation in AKT but reduced in aPKC ($*p = 0.024$, *t*-Student, n = 4 per group). In summary, consumption of a HF/HS diet induces metabolic alterations in dams and higher NTD rates in embryos -independent

of the SR-B1 genotype- which can be prevented by VE supplementation. Further studies will allow us to understand the VE-mediated mechanisms underlying NTD prevention due to HF/HS diet exposure.

#73 Hypoxia-reoxygenation, one of the factors responsible for the alteration of the endocannabinoid system in the placenta

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Introduction: It has been described that the endocannabinoid system (ECS) has several implications in human placentation and that alterations in this system are associated with placental pathologies. We previously demonstrated that the ECS is dysregulated in preeclampsia, with elevated levels of NAPE-PLD and decreased levels of FAAH, the main enzymes responsible for the synthesis and degradation pathways, respectively.

Objectives: Evaluate if the changes in the ECS seen in preeclampsia are also produced by hypoxia-reoxygenation and/or HIF-1a stabilization.

Methods: Placental explants: Term placentas obtained from elective cesareans were cut into 50mg explants and cultured for 6h in DMEM/F-12 + 10% FBS at 37 °C under the following conditions: 6 h in 5% CO_2, 95% air (Control group, Ctrl); 2 h in 5% CO2 2% O2 93% N2 followed by 4 h of re-oxygenation (Hypoxia-reoxygenation group, HR); or 6 h in same gas mixture as Ctrl plus 250 μM $CoCl_2$ (a HIF-1a stabilizing agent, Co group).

Cell culture: BeWo cell line was cultured for 48 h in DMEM/F-12 + 10% FBS at 37 °C in a 5% CO2, 95% air atmosphere in the absence or presence of 25 μM Forskolin. For the hypoxia-reoxygenation group, cells were submitted to two hypoxic events: at 0 h and 24 h.

FAAH and NAPE-PLD mRNA levels were quantified by RT-qPCR. FAAH, NAPE-PLD and HIF-1a protein levels were assessed by Western Blot. Data from 3–4 independent experiments were analyzed using paired *t*-test or ANOVA followed by Dunnett's test.

Results: Explants from HR group showed higher NAPE-PLD mRNA levels, while the Co group did not present any changes. In the case of FAAH, both conditions significantly diminished FAAH mRNA. BeWo cells incubated with forskolin and exposed to HR showed an increase in NAPE-PLD protein levels.

Conclusions: HR explants but not Co presented an ECS profile similar to preeclampsia. Changes detected in the synthesis and degradation pathways are observed mainly in the syncytiotrophoblast.

-Arts effects on embryo and offspring development

#52 Vitrification with cumulus cells: impact on the oocyte and on the subsequent embryo development

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Brief introduction: In order to evaluate maturity and vitrify oocytes, cumulus cells are totally stripped from them. Thawed oocytes are then inseminated by ICSI to solve the potential low fertilization rate through conventional IVF in fully denuded oocytes.

Objective: Assess the impact of leaving cumulus cells in fresh and thawed oocytes, and its consequences on fertilization and embryo development. Moreover, investigate whether the corona radiata has a protective effect on the oocyte during vitrification.

Methods: 718 oocytes were divided into 2 groups: partially denuded and fully denuded. Each group was divided into fresh and thawed, thus forming a total of 4 groups: fresh with cumulus ("group A"), fresh denuded ("group B"), thawed with cumulus ("group C") and thawed denuded ("group D"). Survival and IVF results were compared between groups.

Main Outcomes and Results: Statistically significant differences were found in fertilization rates between group A and group B, with better results in group A ($p = 0.0009$). Regarding the other parameters, no differences were found. No significant differences were found when comparing the survival of group C and group D. Among the group A and group C, no differences were found in the IVF results.

Conclusions: Presence of granulosa cells is vital for good conventional IVF results, especially for fertilization rates. The survival rate in thawed oocytes did not change according to the presence or absence of cumulus cells. This indicates that the corona radiata does not accomplish a protective function. However, their presence does not impact negatively, therefore it is not necessary to denude the oocytes before vitrification, which would benefit maturation, and the presumed possibility of performing conventional IVF. This would decrease the workload in laboratories, and improve the results, since IVF is known as a less invasive and more physiological technique.

#74 Infertility and oocyte cholesterol excess: characterization and subcellular localization of cholesterol puncta in oocytes from hypercholesterolemic mice

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Evidence suggests that disturbances in cholesterol metabolism may hinder female fertility. SR-B1 knockout (KO) female mice are infertile due to oocyte unesterified cholesterol (UC) excess, defective meiotic arrest, and compromised viability. In previous studies, the UC-specific fluorescent dye filipin showed uniform distribution and bright puncta in both WT and KO oocytes, with much stronger fluorescence in SR-B1 KO oocytes. In this work, we evaluated quantitative differences in cholesterol staining between WT and KO oocytes and studied cholesterol subcellular localization in oocytes. Mature oocytes were obtained from adult WT or SR-B1 KO female mice after superovulation. We used filipin staining (0.2 mg/mL) in combination with specific markers for cellular organelles: 200 nM Mitotracker for mitochondria and 100 nM BODIPY for lipid droplets (LD), and anti-CD9 and anti-LAMP1 antibodies (1 : 100) for plasma membrane and lysosomes. Stained oocytes were visualized using confocal microscopy, and 10 z-stacks were registered per cell. Cholesterol co-localization with organelles was analyzed using Manders' coefficient and statistical differences determined using *t*-test. We found that the mean shape, number, and size of cholesterol puncta/oocyte were similar in WT and KO (n = 6 and 8 oocytes, 10 z-stacks each) suggesting a similar subcellular localization in WT and KO oocytes. Cholesterol puncta were 2.17 ± 0.48 (SEM) times brighter in SR-B1 KO vs WT oocytes ($p = 0.04$). Cholesterol co-localization with plasma membrane was low and similar between genotypes (8.0 ± 6.3% in WT vs 2.8 ± 1.8% in KO). Preliminary results in WT oocytes (n = 3) showed partial co-localization of cholesterol puncta with LD and lysosomes (38.4 ± 13.1% and 38.2 ± 7.1%. respectively). By contrast, cholesterol co-localization with mitochondria was lower (4.6 ± 1.6%). Further studies will determine the subcellular localization of cholesterol in KO oocytes and provide information to explain the adverse effect of cholesterol excess in SR-B1 KO oocytes.

#10071 Effect of insulin- transferrin- selenium and metformin on the *in vitro* fertilization of pigs

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Brief introduction: The *in vitro* production of pig embryos is important for both commercial purposes and biomedical studies. It has a low efficiency mainly because *in vitro* maturation (IVM) systems are inefficient. Supplementation of the IVM medium with insulin plus metformin(M) improves pig embryonic development. We demonstrated that insulin-transferrin-selenium (ITS) plus M increases nuclear maturation, decreases oxidative stress, increases glucose consumption and the viability of cumulus cells.

Objective: Our aim was to study the IVF efficiency using ITS plus M during IVM.

Methods: Porcine COC obtained by follicular aspiration from slaughterhouse ovaries were in vitro matured for 44 h in modifed M199, supplemented with hMG and dAMPc during the first 22 h. Oocytes were randomly distributed in four experimental groups containing: ITS (ITS 1 μg/mL), M (M 10-4M), ITS + M and C (no supplement). Then matured oocytes were denuded and subjected to IVF (co-incubated 4 h with refrigerated boar semen 1 × 106 sperm/mL in modified M199). The presumptive zygotes were cultured in NCSU at 39 °C with 5% CO_2, 7% O2 and 100% humidity. After 20 h from the beginning of the IVF, zygotes were fixed in paraformaldehyde 4% for 30 min, washed and stored in PBS-PVA until use. To assess fertilization parameters, zygotes were stained with Hoëchst and mounted for evaluation: penetration (penetrated/inseminated), masculine pronuclear formation (MPN/penetrated), monospermy (only one sperm head or male pronucleus/penetrated), and efficiency of fertilization (monospermic/inseminated). Data were analyzed by Fisher's exact test (significant $P < 0.05$).

Main Outcomes and Results: The group matured with ITS + M showed a significantly higher efficiency of fertilization compared to control (ITS + M 30%, n = 43 vs C 11%, n = 45).

Conclusions: The supplementation of IVM media with ITS + M is a good tool to improve oocyte quality. This is reflected in a higher IVF efficiency.

-Paternal effects on fertility and offspring health

#5 Disruption of paternal circadian rhythm controls metabolic health in male offspring via non-germ cells factors

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Circadian rhythm synchronizes each body function with the environment and regulates physiology. Disruption of normal circadian rhythm alters organismal physiology and increases disease risk. Recent epidemiological data and studies in model organisms have shown that maternal circadian disruption is important for offspring health and adult phenotypes. Less is known about the role of paternal circadian rhythm for offspring health. Here, we disrupted circadian rhythm in male mice by night-restricted feeding and showed that paternal circadian disruption at conception is important for offspring feeding behavior, metabolic health and oscillatory transcription. Mechanistically, our data suggest that the effect of paternal circadian disruption is not transferred to the offspring via

the germ cells, but initiated by corticosterone-based parental communication at conception and programmed during in utero development through a state of fetal growth restriction. These findings indicate paternal circadian health at conception as a novel determinant of offspring phenotypes.

#7 Extracellular vesicles from semen induce tolerance in antigen-presenting cells and promote the generation of regulatory T cells

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A healthy pregnancy requires the maternal immune system to tolerate the fetus. Epidemiological evidence suggests that induction of maternal tolerance to the fetus must occur, at least in part, before pregnancy, during exposure to antigens from semen. Our goal is to investigate how extracellular vesicles from semen (SEV) induce tolerogenic dendritic cells (DCs) which promote the differentiation of regulatory T cells. We used two primary measures of DC function following treatment with SEV: (1) measuring the glycolytic rate and (2) testing whether SEV-treated DCs induced a regulatory phenotype and function in co-cultured T cells. DCs were treated with SEV and glycolytic rate was measured. For generation of Tregs, DCs were loaded overnight with SEV with or without allogeneic antigen, then cultured with CD4+ T cells for 12 days. These T cells (putative Tregs) were cultured with fresh syngeneic labeled CD4+ T cells (responder cells) and stimulated with activator DynaBeads. Proliferation of responders and Treg phenotype was measured by flow cytometry. SEV blocks the ability of DCs to increase the glycolytic rate following a challenge. Culturing T cells with syngeneic SEV-treated DCs increased the fraction of CD25HICD127LO CD4+ T cells, a marker for regulatory phenotype from 31.7% to 58.4%. These putative Tregs decreased the proliferation of responder T cells in a dose-responsive manner ($P = 0.00013$). SEV induce a tolerogenic metabolic profile in DCs. T cells cultured in the presence of DCs loaded with SEV and antigen (1) expressed a classical Treg phenotype (CD25HICD127LO) and (2) suppressed the proliferation of stimulated T cells. Together, this evidence suggests that SEV in semen bias genital antigen-presenting cells to a tolerogenic phenotype. This in turn drives the generation of regulatory T cells. *In vivo*, over many cumulative exposures to semen, this could lead to a pool of Tregs poised to regulate immune responses against the fetus.

#17 Chronic heroin use generates distinct cleavage patterns of tRNA-Gly-GCC in semen

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In addition to their role in protein translation, transfer RNA (tRNAs) can be cleaved into shorter, biologically active fragments called tRFs. The generation of tRFs is highly regulated; alterations in tRF patterns are associated with stress and exposure to toxicants. Recent studies demonstrate a role for tRFs in spermatocytes to cause heritable metabolic disorder. This suggests tRFs are potentially a mechanism of epigenetic inheritance. As yet, the impact of opioid use on tRFs in germline cells has not been explored.

We ran an RNAseq experiment on RNA from highly purified spermatocytes and semen-derived exosomes from men who inject drugs and non-drug using controls. We validated results using droplet digital PCR, with specific reverse-transcription primers for long versus short tRF fragments.

We found that a tRF from Gly-GCC tRNA demonstrated different cleavage in spermatocytes from people who inject drugs (PWID) compared to non-users. Over 90% of reads in non-drug using men mapped to shorter tRFs, while in PWID only 45% did. In contrast, only 4.1% of reads in controls mapped to a longer tRF species, compared to 45.6% in PWID. The long/short tRF ratio was significantly higher in PWID than non-drug users (0.65 vs. 0.14, $p = 0.03$). We also report in exosomes differential expression of a group of small nucleolar RNAs (snoRNAs) that includes, among others, ACA14a, U19 and U3-3.

In summary, in semen samples from PWID, we observed an altered cleavage pattern of tRNA-Gly-GCC compared to that found in non-heroin users. Additionally, in semen-derived exosomes, we reported an altered cargo of snoRNAs in PWID. This study lays the groundwork to investigate whether other tRFs or other epigenetic mechanisms like DNA methylation are changed following opioid use and whether these changes can be reversed by withdrawal or by opioid substitution therapies.

#32 Paternal ethanol consumption affects male offspring intergenerationally

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Ethanol consumption could profoundly affect sperm chromatin and, thus, have a severe impact on reproduction. Previously, we observed that male ethanol consumption increased the sperm rate of decondensation, affecting the kinetics of fertilization. Here, we analyzed the effect of moderate paternal ethanol consumption on both their own reproductive capacity and that of their male offspring (F1). CF-1 male mice were exposed (treated group, T) or not (control group, C) to 15% (v/v) ethanol in drinking water ad libitum for 15 days. Spermatozoa from epididymal cauda were obtained by swim-out to determine sperm oxidative stress and epigenetic marks of histone modifications by immunocytochemistry. Testicular histology was analyzed and the DNA fragmentation was studied by TUNEL assay on both groups. Males from C and T groups were mated with non-treated CF-1 female mice. Sperm from adult male mice of the F1 were obtained by swim-out to determine epigenetic histone modifications. Testicles of F1 mice were analyzed histologically. Male ethanol consumption decreased the size of the lumen of the seminiferous tubules and increased sperm oxidative stress in the T group. We observed a significant decrease of epigenetic marks of histone H3K4me3 in sperm from T group vs. C group. We also detected an increase in TUNEL labeling on the germinal line in testicles from T groups. When we analyzed F1 mice we detected differences in the diameter and thickness of the epithelium of their seminiferous tubules being both significantly minor that those in the C group. Paternal ethanol consumption significantly increased the abundance of epigenetic marks in histones H3K9me and H4K12ac in the spermatozoa of the F1. Altogether, our study provides critical information on the paternal reproductive disturbances, as a consequence of moderate ethanol consumption, and the profound impact they could have on their F1.

#36 Effects of paternal alcohol intake on the neurological health of the offspring

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Previously, we observed that male alcohol consumption affected sperm biochemical parameters and DNA integrity. CF1 male mice were exposed (treated) or not (control) to 15% (v/v) ethanol in drinking water ad libitum for 12 days. Males (treated or control) were mated with untreated females and two-cell embryos were collected and cultured for 7 days. The embryos from treated males presented anomalous inner cell mass and trophoblast morphologies. Other pregnant females were allowed to complete the gestational period. We found that the offspring of treated males were under weighed during the first weeks of life, recovering weight in adulthood when they became over weighed. Also, we detected that the treated mice offspring health status was somehow altered with a modification of the spleen and blood cell populations. The resulting progeny showed lighter brains, and significantly altered physical and behavioral parameters in treated group. Litter from treated males presented a delay in surface righting, taking longer time to do it seven days after birth. The appearance of the hind grasping reflex was also delayed in male and female offspring from treated males. The Open Field test showed that males spent a longer time in the center of the maze than the control group, suggesting they had more anxiety-like behaviors. Also, control group was more socially dominant than treated in male and female offspring. Alterations in dominance parameters, however, were modified by early-life exposure to an enriched environment. Finally, we made a screening for potential modifications in the expression of candidate genes in the medial prefrontal cortex of young adult males from the offspring of treated mice. This revealed elevated expression of Egr1, a gene involved in neural plasticity. Moderate paternal alcohol intake has a detrimental effect on its progeny's social skills, associated with altered expression of mPFC genes involved in neural plasticity.

#37 Paternal programming of metabolic alterations in the placenta

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Paternal exposure to diabetes can lead to the transmission of metabolic disorders in the offspring. Our aim was to evaluate the regulation of lipid metabolism in the placenta of male fetuses (F2) from healthy pregnant rats that were mated with male diabetic rats (F1).

Control (C) and type 2 diabetic male rats (D, diabetes obtained by intrauterine programming, glycemia 140–190 mg/dL) were mated with control female rats. On day 21 of gestation, the pregnant and male rats were euthanized. The placentas, the fetuses and fetal and paternal plasma were obtained for further evaluation.

In the paternal and fetal plasma of D group, the levels of triglycerides and cholesterol were increased ($p < 0.05$ vs C). Fetal weight was increased in D group ($p < 0.05$), and placental weight was similar in both groups. The levels of triglycerides (46%), cholesterol (43%) and free fatty acids (47%) were increased in the placenta of D group ($p < 0.05$ vs C). The mRNA levels of Ppar Alpha and its co-activator Pgc 1 Alpha, and the mRNA levels of Fatp1 and Lipg were increased in the placenta of D group ($p < 0.05$ vs C).

Paternal diabetes programs alterations in the feto-placental lipid metabolism. The intergenerational transmission of these metabolic alterations may lead to adverse consequences to the adult offspring.

#40 Amelirative effects of lutein against cyclosporine-induced testicular oxidative stress, apoptotic and inflammatory flux in male Wistar rats

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Cyclosporine, an immunosuppressive drug commonly used in renal transplant recipients to prevent rejection has been implicated as a male reproductive toxicant. Hence, this study was aimed to evaluate the protective effect of Lutein on cyclosporine-induced testicular oxidative stress, apoptotic and inflammatory flux in male Wistar rats. Sexually matured male Wistar rats (weighing 200 ± 50 g) with six animals in each group were given cyclosporine (40 mg/kg) and/or lutein (30 mg/kg) daily via gavage for 4 weeks. The sperm counts, sperm motility, sperm morphology, daily sperm production (DSP), LH, FSH, testosterone, testicular antioxidant systems: superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total sulfhydryl (T-SH), non protein sulfhydryl (NP-SH), glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px), thiobarbituric acid reactive substances (TBARS) and testicular steroidogenic enzymes (3ß-hydroxysteroid dehydrogenase and 17ß-HSD and spermatogenesis marker enzymes (lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transferase (γ-GT), alkaline phosphatase (ALP), acid phosphatase (ACP), testicular pro-inflammatory cytokines (TNF-a and IL-1ß), nucleic acids and total protein level in the testes, were investigated at the end of the fourth week. Histopathological changes were also evaluated in testis. By the end of the fourth week, lutein was revealed to attenuate impaired sperm indices induced by cyclosporine. In testes, lutein decreased LDH, SDH, ACP and γ-GT and increased CAT, 3ß-HSD, 17ß-HSD and ALP induced alteration by cyclosporine. Moreover, the histopathological evaluation revealed a testicle protective effects by lutein supplementation. These result revealed that lutein co-treatment could ameliorates cyclosporine induced reproductive toxicity which may be possibly through its antioxidant, cytoprotective, antia-apoptotic and anti- anti-inflammatory effects. Therefore, it is suggested that lutein may be used as a promising potent therapeutic agent combined to protect reproductive function from cyclosporine-induced toxicity through reducing oxidative stress, apoptotic and inflammatory responses.

-Maternal effects on pregnancy and offspring health

#6 Environmental enrichment protects against social disruptions and neural marker changes induced by a maternal immune activation (MIA) animal model of schizophrenia

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Brief Introduction: Maternal immune activation (MIA) during pregnancy is associated with neurodevelopmental disorders and long-term gene dysregulation in offspring. Animal models of MIA offer mechanistic ways to explore these long-term changes and opportunities to mitigate them. Environmental enrichment (EE), a clinically relevant intervention, has demonstrated benefit in a lipopolysaccharide (LPS)-induced rat model of MIA. Indeed, our lab has shown EE to protect against disruptions in both social behavior and the hypothalamic-pituitary-adrenal (HPA) 'stress' axis with our LPS MIA model.

Objective: Using a polyinosinic-polycytidylic acid (poly (I:C)) MIA model of schizophrenia, the present study explored whether the benefits of a supportive environment could generalize across immunogens and species. Methods: In the current study female C57BL/6J mice were randomized into standard or EE housing and bred in these conditions. On gestational day 15, mice were challenged with either 20 mg/kg (i.p) of the viral mimetic polyinosinic-polycytidylic acid (poly (I:C)) or equivolume of pyrogen-free saline. After birth, quality of maternal nesting behavior was evaluated. Male and female adult offspring were later assessed on measures of social behavior and neural markers of stress.

Main Outcomes and Results: We show that poly (I:C) challenge led to disrupted maternal care in that MIA dams had nests of lower quality compared to saline treated dams. EE housing protected against this. In offspring, poly (I:C) induced MIA resulted in repetitive behavior and reduced social interest, alongside sex-specific mRNA expression of several ventral hippocampal neural stress markers (e.g., corticotropin releasing hormone (CRH), CRH receptor 1, glucocorticoid receptor and oxytocin receptor). Moreover, MIA males had delayed recovery of plasma corticosterone in response to a novel social encounter. Enrichment housing again protected against these MIA-induced effects.

Conclusions: These data demonstrate that the benefits of EE on social behavior and HPA regulation can generalize across immunogens and species used to induce MIA. Our findings provide further evidence for the viability and utility of EE interventions in maternal and pediatric settings.

#12 Oocyte-embryonic anomalies induced by periconceptional alcohol consumption. Effects of superovulation in a preclinic experimental murine model

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The effects of low-moderate periconceptional alcohol consumption on oocyte-embryonic quality are poorly understood. With the objectives of analyzing the impact of perigestational alcohol intake on organogenic embryo development and potential gametic damage as an etiological factor for embryonic anomalies, we designed a murine experimental model with spontaneous ovulation or superovulation of ethanol administration from before fertilization to early gestation. Mouse adult females (60 days) (CrlFcen:CF1, Bioterio Central de FCEN) were administered for 15 days with ethanol 10% in drinking water (treated females, TF) or water (control females, CF) and were superovulated (5 IU/female of eCG/hCG, CFs and TFs) or not (spontaneous ovulation, CFe and TFe). After mating (day 1 of gestation, D1), ethanol administration continued until D10 in TF (D10-CFs/TFs and D10-CFe/TFs). At D10, TFs had reduced number of implantation sites vs CFs ($p < 0.05$) but were similar in TFe and CFe. The percentage of delayed embryos increased in TFs and TFe vs CFs and CFe, respectively ($p < 0.001$), and the frequency of anomalies in organogenic E10-embryos was higher in TF vs CF ($p < 0.001$). However, the % of early resorptions only increased in TFs vs CFs. To elucidate if embryonic anomalies were associated with ovulatory and gametic alterations, we analyzed the follicular and luteal frequency, (H&E), their proliferation (PCNA IHC) and VEGF expression, and the oocyte quality (Hoescht). The estrous and the follicles/corpus luteum frequencies decreased in TFe vs CFe. After superovulation, the number of ovulated oocytes with abnormal metaphase II, fragmented and parthenogenetically activated was higher in TFs vs CFs ($p < 0.01$, $p < 0.001$). In conclusion, periconceptional alcohol consumption alters ovulation producing nuclear oocyte abnormalities that could lead to early gestational loss and embryonic alterations in organogenesis. The experimental superovulated murine model can constitute a useful tool for detection and monitoring the gametic-embryonic defects induced by drug abuse and/or by assisted reproductive techniques.

#13 Expression of FMR1 isoforms during folliculogenesis in the rat

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The *Fragile X Mental Retardation-1* gene (FMR1) is an example of a gene that has the potential to produce a variety of protein isoforms through alternative splicing. The gene consists of 17 exons and codes for the protein fragile X mental retardation protein (FMRP) who exerts repressive activity on translation by either reversibly stalling

ribosomes or through its interaction with microRNAs. Early studies of *FMR1* revealed the presence of multiple transcript isoforms resulting from exon skipping of exons 12 and 14 and the use of alternative splice sites in exons 15 and 17.

FMR1 is involved, by different mechanisms, in 3 genetic disorders. The absence of FMRP due to an expansion of >200 CGG repeats in the 5'UTR of the gene (full mutation), is responsible for the Fragile X syndrome (FXS) while the premutation state is associated with the Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) and Fragile X-associated Primary Ovarian Insufficiency (FXPOI). Although FMRP is highly expressed in gonads, there is no information available regarding splicing variants in the ovary or its possible implications in FXPOI.

In the present work we aimed at investigating the expression of *FMR1* splicing variants during folliculogenesis in the rat. To obtain ovaries enriched with follicles at different stages, rats were injected either with Diethylstilbestrol (DES) to stimulate the development of early antral follicles (EAF) or with equine chorionic gonadotropin (PMSG) to stimulate the development of preovulatory follicles (PF). Preantral follicles were obtained from untreated prepubertal rats. RNA was extracted from isolated follicles to conduct RT-PCR experiments followed by sequencing. We identified differential expression of the isoforms arising from splicing in exons 12, 14 and 15. Importantly, we described for the first time in a rat tissue the isoform that includes exon 12 and 2 isoforms resulting from splicing events in exons 14 and 15.

#14 Maternal nutrition and regulation of oocyte number and quality

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Maternal nutrition impacts the health of the progeny, but how it regulates the number and quality of the oocytes is uncertain. As oocytes in mammals are formed in the fetal ovary, understanding the signaling pathways or factors that determine oocyte number or quality is challenging. To overcome this, we use Caenorhabditis elegans as a model, where oocyte development during meiosis I occurs in adult gonad. In C. elegans, presence of food triggers insulin-like receptor signaling via RAS-ERK pathway to drive meiotic prophase I progression and oogenesis and its absence halts oogenesis by inactivation of this signaling pathway. Thus, availability of nutrients is directly linked with oocyte numbers and quality; the mechanisms remain unknown. Similar to nutrient deprived animals, genetic mutants with loss of ERK activation also display lower oocyte number even in the presence of nutrition. Conversely, increased ERK activation, through RAS gain-of-function allele, results in production of increased oocyte number. In both cases, the oocytes are of poor quality, as they result in embryonic lethality. Here, we show that RAS/ERK pathway phosphorylates meiotic chromosome axis protein HTP-1 at serine-325. Phosphorylated HTP-1(S325) accumulates in early-mid pachytene stage of germ cells in an ERK-dependent manner, which is necessary for synaptonemal complex extension and maintenance. Lack of HTP-1(S325) phosphorylation results in chromosomal asynapsis, persistence of DNA double strand breaks, aneuploidy and overall reduced oocyte number and quality. While removal of HTP-1 phosphorylation suppressed increased oocyte number in RAS gain-of-function animals, restoring HTP-1 phosphorylation overcome oocyte loss in the ERK loss-of-function mutants. Together, these data suggest that threshold ERK activation coordinates chromosomal behavior via HTP-1 phosphorylation, with meiotic progression to produce healthy oocytes in favorable nutrient-enriched environment. Given the conserved nature of meiosis and RAS-ERK pathway, we propose that maternal nutrition might play a critical role in regulating oocyte number and quality.

#18 Effect of maternal diets enriched in sunflower or chia oil on mTOR pathway in the decidua of diabetic rats during early postimplantation

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Embryo development defects induced by maternal diabetes may start at early pregnancy when histotrophic nutrition occurs through the decidua. Leukine inhibitor factor (LIF), insulin growth factor binding protein 1 (IGFBP1) and matrix metalloproteinases (MMPs) participate in decidualization and development. Mammalian target of rapamycin (mTOR) pathway senses nutrient availability and may be regulated by polyunsaturated fatty acids (PUFAs).

Aim: To evaluate the effect of diets enriched in sunflower and chia oil (enriched in n-6 and n-3 PUFAs respectively) on mTOR pathway, LIF and IGFBP1 gene expression and MMPs activity in the decidua of diabetic rats at day 9 of pregnancy.

Methods: pregestational diabetes was induced in Wistar rats by streptozotocin administration (50 mg/kg). On days 7, 8 and 9 of pregnancy diabetic rats received a standard diet or diets enriched in 6% of sunflower or chia oil. In the decidua of 9-days-pregnant rats we measured mRNA levels of LIF, IGFBP1 and mTOR by RT-qPCR, protein levels of 4EBP1, rpS6 and AKT (proteins downstream mTOR pathway) by Western Blot and MMPs activity by in situ zymography.

Results: The mRNA levels of LIF, IGFBP1 and mTOR were reduced in the decidua of diabetic rats ($p < 0.001$, 71%; $p < 0.05$, 34%; $p < 0.05$, 54%; respectively). Both diets prevented the reduced IGFBP1 mRNA levels. Sunflower-enriched diet prevented the reduced LIF expression. Phosphorylated protein levels of 4EBP1, rpS6 and AKT were reduced in the diabetic group ($p < 0.05$, 27%; $p < 0.05$, 40%; $p < 0.05$, 14%; respectively), with no changes in total protein levels. PUFA-enriched diets restored the phosphorylated/total levels ratio of these proteins. MMPs activity was reduced in the decidua and ectoplacental cone in diabetic rats ($p < 0.05$), an alteration prevented by the PUFAs-enriched diets.

Conclusion: Our results suggest impairments in decidualization and histotrophic nutrition regulation at early postimplantation in diabetic rats, which can be prevented by maternal diets enriched in PUFAs.

#21 Glyphosate and a glyphosate-based herbicide induce fetal growth retardation in second-generation rat offspring

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Epidemiological evidence indicates that maternal exposure to pesticides is associated with stillbirth, altered birth weight or congenital defects. Glyphosate-based herbicides (GBHs) are the pesticides most applied worldwide, and the effects of commercial formulations and its active ingredient glyphosate (Gly) on reproductive health of present and future generations constitute a matter of global interest. We aimed to evaluate whether in utero and lactational exposure to Gly or a GBH induce growth alterations or congenital anomalies in second-generation (F2) offspring. Gly or a GBH (2 mg of glyphosate/kg/day) was administered to F0 pregnant rats through food from gestational day (GD) 9 until weaning. The serum levels of Gly were determined in F0 dams on GD22 and lactational day 21 by UHPLC-MS/MS. Sexually mature F1 females became pregnant and on GD19, F2 fetuses were removed. Morphometric features of F2 offspring were examined: fetal weight, length and morphology, placental weight and placental index. Glyphosate serum levels in F0 dams were similar along the treatment, however, higher levels of Gly were detected in GBH-exposed dams ($p < 0.05$). F2 fetuses from Gly and GBH-exposed groups exhibited decreased fetal weight and length. Higher occurrence of small for gestational age (SGA) F2 fetuses was observed in GBH (57.5%) and Gly (45.4%) groups in comparison to controls (22%), denoting a delayed growth. The relative risk of being SGA was 2.62 [95% CI (1.70, 4.02); $p < 0.001$] for GBH F2 fetuses and 2.06 [95% CI (1.33, 3.20); $p < 0.001$] for Gly F2 fetuses. Although no changes in placental weight were detected, an increase in placental index was observed in Gly group. No structural congenital anomalies were observed. Both Gly and GBH induced multigenerational effects

evidenced by fetal growth retardation. Differences between GBH and Gly effects could indicate particular mechanisms of action of the commercial formulation and active ingredient.

#22 Perinatal exposure to glyphosate or a glyphosate-based formulation disrupts hormonal and uterine milieu during the receptive state in rats

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Glyphosate (Gly) is the active ingredient of all glyphosate-based herbicides (GBHs), which are the most widely applied herbicides worldwide. Nowadays, the safety of Gly and its formulations remains to be a controversial issue. We showed that a GBH formulation administered during gestation and lactation induces subfertility in F1 female rats due to a decrease in embryo implantation. Here, we sought to investigate the effects of perinatal exposure to a GBH or Gly on female fertility, and the hormonal and uterine milieu during the preimplantation period. F0 pregnant rats orally received a GBH or Gly in a dose of 2 mg of glyphosate/kg/day from gestational day (GD) 9 until weaning. F1 females were evaluated to determine the reproductive performance on GD19; and the sex steroid serum levels, the expression of estrogen receptor alpha (ERa), progesterone receptor (PR) and implantation-related genes on GD5 (preimplantation period). GBH and Gly induced preimplantation losses in F1 rats. GBH and Gly groups exhibited higher 17ß-estradiol serum levels (Control: 21.40 ± 2.61 pg/mL; GBH: 30.41 ± 0.93 pg/mL; Gly: 30.41 ± 0.61 pg/mL) without changes in progesterone. Both compounds increased the uterine ERa protein expression: while GBH induced a significant increase in ERa expression in the subepithelial stroma, Gly did in the gland compartment. No differences were detected for ERa at mRNA level between the experimental groups; and only Gly decreased PR mRNA expression. Also, GBH and Gly downregulated Hoxa10 (*$p < 0.05$) and Lif (*$p < 0.05$) genes, with no difference in Muc1 and Areg expression. To conclude, perinatal exposure to a GBH or Gly disrupted critical hormonal and uterine molecular targets during the receptive state, possibly associated with the implantation failures. Overall, similar results were found in GBH- and Gly-exposed rats, suggesting that the active principle might be the main responsible for the deleterious effects.

#26 Maternal environmental enrichment promotes vascular remodeling at the maternal-fetal interface during early gestation in mice

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Implantation-related events are crucial for pregnancy success. In particular, defects in vascular remodeling at the maternal-fetal interface are associated with many obstetric pathologies. Maternal lifestyle affects the development of pregnancy. Physical activity and therapies oriented to reduce stress improve pregnancy outcomes. In animal models, environmental stimulation and enrichment are associated with enhanced well-being, cognitive function and stress resilience. In this study we investigated whether exposure to an EE regulates crucial events during early gestation at the maternal-fetal interface. Therefore, pregnant BALB/c mice were exposed to an EE that combines non-invasive stimuli from the sensory pathway with voluntary physical activity. Reproductive performance was evaluated on day 7 and day 15 of gestation and vascular adaptation parameters at the maternal-fetal interface were analyzed on day 7 of gestation. We observed that mice exposed to an EE presented higher reproductive efficiency than control animals when day 15 of gestation was analyzed (80% vs 45%). However, no differences were detected between the groups when day 7 was studied (75% vs 70%). This suggests that exposure to an EE prevents embryo loss. Furthermore,

exposure to an EE increased the cross-sectional length of the uterine artery and decreased the wall: lumen ratio of the mesometrial decidual vessels, suggesting that it promotes vascular remodeling of the implantation sites. Moreover, nitric oxide synthase activity and inducible nitric oxide synthase expression were increased in EE-housed females' implantation sites. Also, an increase in prostaglandin F2a production and endoglin expression was detected in the EE group. No differences in the histological structure of the implantation sites were observed among the groups. In conclusion, the exposure of pregnant females to an EE regulates uterine physiology, promoting vascular remodeling during early gestation. These adaptations might contribute to preventing embryo loss later in gestation. Our results highlight the importance of the maternal environment for pregnancy success.

#27 Early exposure to high sucrose diet leads to deteriorated ovarian health

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Metabolic syndrome (MS) is correlated with disorders such as polycystic ovary syndrome (PCOS). Although the consumption of a carbohydrate-rich diet is related to MS, it is still unclear whether this diet leads to ovarian dysfunction and PCOS. We investigated the influence of a sucrose-rich diet (HSD) on the ovaries of Wistar rats and the correlation between high consumption of sugary drinks and the prevalence of PCOS in women. Wistar rats received a standard diet (CTR, n = 8) or HSD (HSD, n = 8) from postnatal day 21 to 120. The animals were evaluated weekly to calculate food intake, energy efficiency and weight gain. The onset of puberty and the estrous cycle were monitored daily. Biochemistry, organ morphometry and ovarian histology were performed during euthanasia. The multiple linear regression analysis of fixed effects was performed using data from Brazilian states (459 state-year observations), to test the correlation between the consumption of drinks sweetened with sugar and the prevalence of PCOS. HSD animals were not obese, but had accumulated adipose tissue, hyperglycemia and insulin resistance when compared to CTR. The HSD rats entered puberty before the CTR and the ovaries of HSD animals showed an increase in the number of atrial antral follicles and cystic follicles, which were correlated with hypertrophy of periovarian adipocytes. Finally, there was a positive correlation between the consumption of sugary drinks and the prevalence of PCOS in women. Ingestion of HSD leads to ovarian dysfunction in rats and may be correlated with PCOS in women, suggesting that these changes may lead to public health problems. Therefore, we reinforce the harmful impact of HSD on the ovarian system and suggest that reducing sugar intake may be beneficial for ovarian health.

#28 Evidence for resveratrol modulating inflammatory mediators of maternal immune activation

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Maternal immune activation (MIA) produces structural, metabolic and epigenetic changes in the fetus associated with increased risk for various neurodevelopmental and neurodegenerative disorders. The hallmark of brain neuroinflammation is the activation of microglia, one of the main effectors of the innate immune response in the CNS. Excessive microglial activation leads to CNS damage due to excessive production of pro-inflammatory mediators, like interleukins, MMP, PGE2 and ROS.

It has been demonstrated that resveratrol has anti-inflammatory, antioxidant properties that translate into neuroprotective effects in adults.

Objectives: To study the possible neuroprotective effects of resveratrol in the fetal CNS in a model of MIA induced by bacterial lipopolysaccharide (LPS) and to determine the pro-inflammatory mediators involved in the modulation of the response.

Resveratrol was administered to Balb/c females on gestational day 15, which were then exposed or not to LPS. After LPS administration, maternal sera and amniotic liquid were collected to evaluate the expression of the proinflammatory cytokine IL-6 and MMP2 and MMP9. Additionally the brain of the offspring were collected to evaluate the expression of COX2, the primary source of inflammatory PGE2 synthesis.

Results: Preliminary results show that the LPS-triggered MIA induces IL-6 in the mother sera and in the amniotic fluid ($p < 0.05$ respectively) while resveratrol prevents this effect. The same effect we observed in the evaluation of the COX2 expression. On the other hand, we observed that LPS decreases MMP2 while Resveratrol induces MMP9 in the amniotic fluid.

Conclusion: Maternal immune activation increases IL-6 production in peripheral blood and amniotic fluid. Furthermore, MIA also increases COX2 expression in fetal brains.

#31 Maternal circulating microRNA profiles reflect correlates of placental function and fetal growth throughout normal human pregnancy

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Background: Pregnancy-associated microRNA (miRNA) clusters can be detected in maternal plasma, but their potential as non-invasive biomarkers of pregnancy evolution has not been determined yet. The aim of our study is to determine if circulating miRNAs throughout pregnancy express distinct populations of miRNAs highly expressed in the placental tissue, and if their expression is associated with fetal growth estimates.

Materials and methods: Longitudinal large-scale profiling of circulating miRNAs at three progressive stages during pregnancy and one stage after birth (n = 8) were obtained and contrasted with the corresponding profiles derived from age-matched non-pregnant women (n = 10). miRNA belonging to the C14CM and C19CM families, known for being involved in trophoblast differentiation and function, as well as miRNAs most prominently expressed in placental tissue (upper quartile; 25%) were compared between pregnant and non-pregnant women using nonparametric and numerical methods. A robust signature of circulating miRNAs associated with indicators of fetal growth (femur length and estimated fetal weight) was identified and used to assess their overlap with placental-associated miRNAs populations.

Results: miRNAs belonging to the C19MC family were found collectively up regulated in blood plasma throughout pregnancy ($p < 4 \times 10^{-4}$) but not after birth, when compared with non-pregnant controls. By contrast, miRNAs belonging to the C14MC and miRNAs most prominently expressed in placental tissue were collectively downregulated ($p < 4 \times 10^{-5}$ and $p < 0.02$, respectively) in the blood plasma of pregnant women. Furthermore, C19CM miRNAs were significantly enriched in fetal growth-associated circulating miRNAs ($p < 1 \times 10^{-6}$).

Conclusion: Our results demonstrate the existence of temporal changes of specific c-miRNAs associated to both placental tissue and fetal growth, supporting the idea of peripheral miRNA profiling as a potential tool for the non-invasive monitoring of pregnancy evolution.

#38 The influence of cabergoline on the offspring phenotype of human chorionic gonadotropin (hCG)- secreting female mice: does mother's milk make the difference?

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Transgenic female mice expressing human chorionic gonadotropin-ß (hCGß+) produce elevated levels of hCG, prolactin and progesterone, show precocious puberty, are infertile and develop pituitary tumors. We have previously demonstrated that a short-term treatment of juvenile hCGß+ females with the dopamine agonist cabergoline normalizes the phenotypic changes of hCGß+ females. Even more, the treatment prevented phenotypic alterations on the transgenic offspring. The aim of this study was to determine if the cabergoline treatment has its effect during pregnancy and/or lactation. Two groups of 2-month-old wild-type (WT) females were mated with hCGß+ males: (1) Six-week-old WT females pretreated with cabergoline (500 µg/kg, ip), every other day for one week (WT-CAB mothers); (2) WT females without treatment (WT- mothers). Offspring from each mother was exchanged at birth and analyzed at three weeks of age. Transgenic offspring from WT-CAB mothers that ingested milk from WT mothers showed phenotypic alterations as exhibited in hCGß+ females, in terms of vaginal opening and increased uterus weight, as indicators of precocious puberty. On the other hand, the phenotype of transgenic offspring from WT mothers that received milk from WT-CAB mothers was normalized in terms of vaginal opening, uterus weight and ovarian gene expression of Lhcgr, Cyp11a1, Cyp17a1 and Cyp19a1 (qPCR). To analyze if the milk makes the difference, another group of WT females previously mated with hCGß+ males was treated with cabergoline during lactation from day 1 after birth for one week (0.1 µg/kg ip, every other day). Female transgenic offspring also showed a normalized phenotype at 3 weeks of age. These results suggest that cabergoline has an impact on the offspring during the lactating period and protects them from the phenotypic alterations induced by hCG hypersecretion. The molecular mechanisms involved in this phenomenon remain to be investigated.

#41 *In vivo* evaluation of estrogenic effects of dietary supplement Hops

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Hops is used as a suspected safe alternative to hormone replacement therapy for menopausal symptoms relief. Hops contains, among others, the phytoestrogen 8-prenylnaringenin (8-PN) and xantohumol (XN), which can be metabolized to 8-PN. XN acts as a cancer chemopreventive agent.

We aimed to evaluate the estrogenic properties of hops and knockout hops (KO-hops) (reduced in XN and consequently in 8-PN) using the uterotrophic assay.

Seven weeks old female Wistar rats were bilaterally ovariectomized. After fourteen days, rats were treated for three days with 17ß-estradiol (E2: 4 µg/kg bw/day) or fed with the vehicle (CON), hops or KO-hops at 8, 40 and 200 mg/kg bw/day. Animals were sacrificed 24 h after the last treatment day. The uterus was removed, weighed and processed for histology and mRNA extraction.

As expected, the relative uterine weight (rUW) and luminal epithelial cell height (LECH) were increased by the positive control E2 respect to CON ($p < 0.05$). The rUW was similar between hops, KO-hops and CON groups. An increase in LECH was shown in hops40 compared to CON ($p < 0.05$). E2 and KO-hops8 induced cell proliferation in the luminal epithelium compared to CON ($p < 0.05$). The mRNA expression of estrogen receptor a (Esr1) and complement C3 (C3) was downregulated and upregulated, respectively by E2 treatment ($p < 0.05$). Esr1 and C3

mRNA were changed neither by hops nor by KO-hops with respect to the CON. The mRNA expression of progesterone receptor was similar between all evaluated groups. In general, no uterotrophic effects were observed in the endometrium in response to hops extracts, independently of their composition.

In conclusion, the absence of uterine estrogenic effects provides evidence for the safety of both extracts. These preliminary results encourage us to study hops extracts for its chemopreventive properties, although more experiments are needed.

#43 Prenatal hyperandrogenization alters the expression of the main lipogenic genes in gonadal adipose tissue in a female rat model

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Prenatal androgen exposure induces fetal programming, which, together with genetic and environmental factors during intrauterine life, lead to the appearance of polycystic ovary syndrome (PCOS) even during early ages. However, it has been reported that PCOS women, even lean ones, have aberrant adipose tissue morphology and function. This study aimed to evaluate the effect of prenatal hyperandrogenism on the main lipogenesis-related genes in gonadal adipose tissue of adult rats (90 days of age).

Pregnant rats were injected with 1mg of testosterone, and the Control group was obtained by injections of vehicle. The female offspring of prenatally hyperandrogenized (PH) and Control groups were characterized according to the estrous cycle as irregular ovulatory phenotype (PHiov) and anovulatory phenotype (PHanov). We quantified, by qPCR, the gene expression of the main enzymes involved in lipogenesis (Acaca, Acacb, Fas). Moreover, the protein expression of PPARg and PPARa (crucial modulators of lipid metabolism) were studied by Western Blot.

We found that Acaca, Acacb, and Fas mRNA levels were increased in the PHiov group ($p < 0.05$), and only the gene expression of Acaca was decreased in the PHanov group ($p < 0.01$) if compared to the Control group. It is important to point out that Acac synthetizes Malonyl-CoA, which is the limiting factor in lipogenesis. Regarding PPARa, which promotes lipid utilization, its protein levels were low in both PH groups ($p < 0.01$). However, no differences were found in PPARg protein expression between all groups ($p > 0.05$).

We conclude that hyperandrogenization in prenatal developmental periods could play an important role in the deregulation of gonadal adipose tissue during adult age. This was evidenced by alterations in lipid metabolism that involved the expression of the main lipogenic genes and PPARa levels. Thus, this work demonstrated that gonadal adipose tissue would be differentially altered in both PH groups in a phenotypic-dependent manner.

#46 Intrauterine growth restriction impacts folliculogenesis in young female pigs

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Intrauterine growth restriction (IUGR) may impact health in adulthood, as it predisposes to several diseases. Pigs have been used as animal models to study the adverse effects of IUGR, although there is little information on its consequences on the reproductive system, particularly in females. We investigated, through histomorphometrical analyzes, follicular development in normal birth weight (NBW) and intrauterine growth restricted (IUGR) female pigs (gilts) at three different ages: birth, 100 days, and 150 days. Thirty-six littermate gilts were selected at birth and allocated to two experimental groups, according to birth weight: IUGR (0.8–1.0 kg) and NBW (1.4–1.7 kg). Euthanasia was carried out at three stages: birth, 100 days, and 150 days of age (n = 6 littermate pairs per age). Subsequently, body and organs weights were measured, and the ovaries were collected, fixed by immersion in paraformaldehyde and processed to obtain histological sections and determine the follicle population counts. IUGR

newborns presented lower liver and brain weights, and higher brain/liver weight ratio ($P < 0.05$), providing evidence of intrauterine growth restriction. IUGR females presented lower body weights at 100 and 150 days of age ($P < 0.05$), and slightly smaller ovaries at 150 days ($P = 0.06$). Additionally, they also presented greater number of primordial follicles at 100 days, and lower number of atretic follicles at 150 days ($P < 0.05$). A discrete commitment of recruitment rate at 100 days was also observed in IUGR gilts ($P = 0.07$). Thus, IUGR impacts folliculogenesis due to a reduction in the rate of follicular recruitment and in the number of atretic follicles, which may reflect on subsequent litter size.

#47 Implantation failure caused by uterine glandular dysfunction in rats exposed to glyphosate or a commercial glyphosate-based formulation

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Glyphosate-based herbicides (GBHs) are the most widely applied pesticides in the world. Glyphosate (Gly), the active ingredient of all GBHs, is combined with other chemicals known as co-formulants to enhance herbicide action. Little is known about the contribution of co-formulants to the toxicity of herbicides. In addition, evidence is not conclusive whether the adverse effects are caused by Gly or GBH. Recently we have shown that perinatal exposure to either Gly or GBH decreased the number of implanted embryos in rats. In the present work, we sought to investigate whether implantation failures in Gly and GBH exposed female rats are related to alterations in endometrial gland function. Pregnant rats (F0) were exposed to Gly or GBH through food, in a dose of 2 mg of glyphosate/kg/day, from gestational day (GD) 9 until weaning. Sexually mature F1 females became pregnant and were euthanized on GD5 (pre-implantation period) to assess the number of glands in uterine samples and the expression of molecules that regulate uterine gland function and implantation such as, leukemia inhibitory factor (Lif) assessed by qPCR, and Forkhead box A2 (FOXA2) and b-catenin evaluated by immunohistochemistry. A lower number of uterine glands in Gly and GBH-exposed groups was detected in relation to the control. Both Gly and GBH exposure decreased the expression of Lif. Also, FOXA2 and b-catenin expression levels were downregulated in the glandular compartment in both exposed groups. In conclusion, perinatal exposure to Gly or GBH decreased the number of glands in the pre-implantation uterus and downregulated molecules with key roles for endometrial gland activity and implantation. These morphological and molecular alterations suggest that uterine gland dysfunction might be a mechanism of Gly- and GBH-induced implantation failures. Importantly, Gly and GBH induced similar changes suggesting that both compounds may act through similar pathways.

#48 Prenatal androgen excess and intergenerational actions

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Prenatal androgen excess is considered as one of the main factors contributing to the development of PCOS features in first-generation (F1) female offspring. However, the intergenerational effects in offspring of mothers with PCOS remain unclear.

In previous studies, we found that prenatal hyperandrogenism leads to biochemical hyperandrogenism and insulin resistance in the F1 female generation. Here, we aimed to evaluate whether prenatal hyperandrogenism affects F1 female's pregnancy outcomes and fetal growth of the second-generation (F2) pups.

Pregnant Sprague Dawley rats (F0) were injected with testosterone or vehicle. Then, a prenatally hyperandrogenized (PH) and a control (Ctl) group were obtained, respectively. The anogenital distance (AGD) of PH and Ctl F1-females was measured and they were mated with control males. Body mass was followed during pregnancy. A group of pregnant rats was euthanized on day 14 of gestation (GD14). The number of implantation sites and fetus

weight were determined. Another group underwent normal parturition and anthropometrical measurements (weight, length, and AGD) were made in F2 offspring at day 6 of post-natal life (PND6).

We found no differences between groups in weight gain during pregnancy. The number of implantation sites at GD14 was decreased in the PH phenotype ($p < 0.01$), but no alterations were observed in the number of embryo resorption sites ($p > 0.05$). The body mass of F2 pups at GD14 and PND6 was decreased in offspring of PH mothers ($p < 0.01$). Moreover, the AGD to body length ratio was decreased in female pups at PND6 from PH mothers ($p < 0.01$).

We conclude that prenatal androgen exposure at a F0 generation altered the F1 reproductive capacity. The F2 development was also affected, as it was observed an altered body mass and length in F2 pups. However, further studies are needed to determine the role of the dysfunctions associated with PCOS on pregnancy complications and embryo development.

#51 Effect of gestational obesity on maternal-fetal DHA transfer over fatty acid metabolism in fetal liver

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Maternal obesity is related to a depletion of docosahexaenoic acid (DHA), which can cause an imbalance in the oxidation of fatty acids (FA) favoring lipid accumulation in the fetal liver. The aim of this study was to elucidate the effect of DHA supplementation on placental transfer of DHA and key molecules involved in hepatic FA metabolism in fetus of obese mothers. Eight weeks old C57BL/6 female mice were fed with a control diet (CD, 14.9% Kcal fat) (n = 13) or an obesogenic diet (HF, 45% Kcal fat) (n = 15) for 4–6 weeks previous pregnancy until gestational day (GD) 17.5. Seven HF and seven CD mice were supplemented with DHA (100 mg/Kg) orally from GD 6.5 until 16.5. On day 17.5 animals were fasted for 4 hours, subjected to oral glucose tolerance test, and then anesthetized and euthanized. Placentas and fetal liver were collected and weighed. Gene expression was determined by qPCR for Mfsd2a, Lipg, Scl27a4, Cd36 and Cpt1 in placenta and for Cpt1, Ppara, Acsl, Acox, Fas, Acaca and Srebf in fetal liver. Statistical differences were determined by Two-Way ANOVA and Sidak post-test ($p < 0.05$ was considered significant). Male fetuses from HF mothers showed a lower gene expression of Mfsd2a and Lipg in placenta and Cpt1 and Acsl in fetal liver compared to control group ($p < 0.05$). DHA supplementation mainly showed effects in female fetus increasing body weight and placental efficiency in those from HF mothers, and the mRNA expression of Cpt1 and Ppara in liver of fetus both dams fed with HF and CD ($p < 0.05$). However, DHA causes an increase of liver weight and placental mRNA expression of Lipg in male fetuses ($p < 0.05$). Data suggest that gestational obesity and DHA supplementation have differential effects on placental function and the expression of genes encoding for FA metabolism enzyme fetal liver in a sex specific manner.

#54 Evaluation of ovarian dynamics and follicular growth in female mice exposed to Bisphenol A (BPA)

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Bisphenol A (BPA) monomer is the primary component of polycarbonate plastics and epoxy resins used in the production of bottles, food packaging, medical devices, dentist resins and other materials. Due to its structural characteristics, BPA acts as an endocrine disruptor and is associated with breast, endometrium, ovary, prostate, testis and thyroid cancers. In order to evaluate BPA impacts on ovarian dynamics and follicular growth, 4 weeks old C57BL/6 mice were divided in three groups and maintained under controlled conditions of light and temperature. The control group received water ad libitum for a period of six weeks, while DMSO and BPA groups received water enriched with dimethylsulfoxide (200 mg/L) and BPA (200 mg/L), respectively, during the same period of time. Following euthanasia, ovaries from animals of all experimental groups were prepared for histological analysis and follicular and corpora lutea quantification. Follicles and corpora lutea numbers were obtained and follicular recruitment and atresia rates were calculated. For all analyses, no significant differences ($p > 0.05$) were found in the number of follicles and corpora lutea and in the follicular recruitment and atresia rates between control, DMSO and BPA groups. Based on these results, we suggest that BPA does not impact neither ovarian dynamics nor follicular growth. However, further studies should be performed to investigate possible changes in the patterns of secretion and responsiveness to gonadotropins, considering that BPA is related to endocrine deregulation in different organs.

#56 Maternal treatment with Butyrate prevents metabolic impairments in the fetuses from overweight rats

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Maternal obesity programs metabolic abnormalities in fetuses that precede a high susceptibility to the development of fatty liver later in life. Butyrate, a short chain fatty acid product of fiber metabolism from intestinal microbiota, improves lipid homeostasis and prevents inflammation.

Aim: To evaluate whether maternal administration of butyrate during gestation ameliorates lipid metabolic anomalies in maternal liver, placentas and fetuses from overweight rats.

Methods: Female rats were fed standard (CT rats) or saturated fat-rich-diet (FD rats) for 8 weeks and mated with control males. Sodium butyrate (3%) or vehicle was orally delivered daily during gestation (FDB rats). At gestational day 21, all rats were euthanized. Fetuses, placentas and maternal and fetal liver were explanted and weighed. Triglyceridemia was assessed by colorimetric assays. Placenta, maternal and fetal liver lipid levels of triglycerides (TG), Free Fatty Acids (FFA), Cholesterol (Ch) and Cholesterol Esters (ChE) were assessed by TLC.

Results: FD mothers showed hypertriglyceridemia (35%, $p < 0.05$ vs. CT), alteration prevented by the administration of butyrate (30%, $p < 0.05$ vs. FD). Lipid overaccumulation in livers from FD mothers (TG: 300% and Ch E: 150%, $p < 0.001$ vs. CT) persisted in FDB rats (TG: 440% and Ch E: 205%, $p < 0.001$ vs. CT). Also, FD placentas lipid overaccumulation (Ch: 101%, FFA: 158%, TG 102% and Ch E: 59%, $p < 0.05$ vs. CT) persisted in FDB group. On the other hand, butyrate prevented the macrosomia observed in the FD group (7%, $p < 0.05$ FD vs. CT), (7%, $p < 0.05$ FDB vs. to FD). Lipid overaccumulation in FD fetuses (TG: 139% and Ch E: 145%, $p < 0.05$ vs. CT) was prevented by butyrate (TG: 50% and Ch E: 45%, $p < 0.05$ vs. FD).

Conclusions: Maternal oral administration of butyrate prevents the increase in maternal triglyceridemia, fetal macrosomia and liver lipid overaccumulation. The prevention of these alterations will probably improve the metabolic program of the offspring.

#57 Intermittent fasting associated to caffeine intake during three reproductive cycles promotes body and uterine weights losses in adult female swiss mice

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Intermittent fasting (IF) is based on high food deprivation for a certain period of time. Some protocols allow the consumption of coffee and teas that contain caffeine in their composition. Little is known about the effects of IF combined with caffeine intake on female reproduction. The present study aimed to evaluate the biometry of ovaries and uterus of female Swiss mice exposed to IF associated with caffeine intake during three reproductive cycles (CEUA/UFMG; nº 113/2020). Females were owed in 6 experimental groups (n = 5/group): control (CC feeding ad libitum); caffeine (T1); intermittent fasting 12 hours (T2); intermittent fasting for 18 hours (T3); intermittent fasting 12 hours + caffeine (T4) intermittent fasting 18 hours + caffeine (T5). Animals received 120 mg/kg/day of caffeine via gavage for two weeks, were weighed daily until euthanized. Animals from the T5 group showed lower body weight compared to their counterparts ($P < 0.05$). Additionally, T4 females were lighter compared to T2 ($P < 0.05$). However, ovarian weight was not affected by different nutritional treatments ($P > 0.05$). Although the ovarian weight was not affected by the previous nutritional regimens, the uterine biometrics for the T4 group was reduced, when compared groups to the CC and ($P < 0.05$). In conclusion, exposure to IF associated with caffeine intake can promote a reduction in body and uterine weight, without changing the biometrics of the ovary.

#58 The offspring of women with pregestational obesity have an increase in umbilical cord blood hematopoietic progenitor cells

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Pregestational obesity (PGO) is associated with an altered immune function in the offspring, mainly compromising the monocyte's response. However, if the hematopoietic stem and progenitor cells of these newborns are compromised has not been described.

Aim: To study the phenotype of neonatal monocytes and their hematopoietic progenitors in umbilical cord blood (UCB) of the offspring of PGO and normal-weight (NW) women.

Methods: UCB samples were collected from the neonates from NW and PGO women. Counts of Hematopoietic Stem Cells (HSCs; lin-CD34+CD45low), Hematopoietic Progenitor Cells (HPCs; Myeloid: CD38+CD7-, Lymphoid: CD38+CD7+ and Common Lymphoid, CL: CD38-CD7+) and Monocyte (Mo) subtypes (Classic: CD14+CD16lowCD64+, Intermediate: CD14+CD16+CD64+, Non-classic: CD14lowCD16+CD64+) were analysed by flow cytometry (BD-FACS Canto II).

Results: Twenty-one NW (BMI > 18.5 = 25 kg/m^2) and eleven PGO (BMI = 30 kg/m^2) UBC samples were included in this preliminary study. An expected low count of CD34+ HSCs and HPCs were found in all UCB samples (0.3% of leukocytes), with a higher count in PGO. The CD34+ population was mainly myeloid HPCs (~90%), with a higher count in the PGO group. Lymphoid and CL HPCs were less frequent phenotypes in the CD34+ population

(~8% and 0.1%, respectively) with a lesser count in the PGO group. Both myeloid and lymphoid HPCs express HLA-DR, corresponding to a late phenotype. UCB Mo counts were in the range of 6799–31,569/μL, with a lower count in the PGO group. The classic monocyte subtype was the most frequent phenotype (~90%), and the intermediate (3.1%) and non-classical (0.3%) subtypes were less represented in UCB. There were no differences in monocyte ś subtypes among groups.

Conclusion: An increase in CD34+ Hematopoietic Stem and Progenitor cells and Myeloid HPCs, and a decrease in Lymphoid HPCs could underlie the onset of immune dysfunction in the offspring of PGO women.

#59 Effects of Curatella americana aqueous extract on early diabetic pregnancy in laboratory animals

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Introduction: Diabetes mellitus is a syndrome that causes risk for pregnant women and can compromise the descendants. Alternative therapies are vastly used to treat diabetes, such as medicinal plants. Curatella americana is one of plants indiscriminately used, however its impact during pregnancy is unclear.

Objective: To evaluate the C. americana extract effects on the pre-embryos development of diabetic rats.

Methods: At birth, female newborn rats received beta-cytotoxic agent to induce diabetes. At adulthood, an oral glucose tolerance test was performed to confirm diabetes status. Next, the rats were mated and randomized into four experimental groups (n = 5 animals/group): (1) nondiabetic pregnant rats given water (C) or (2) given C. americana aqueous extract (CT); (3) diabetic pregnant rats treated with water (D) or (4) given C. americana extract (DT). The C. americana leaf aqueous extract was orally (gavage) administered. On gestational day 4 (GD4), the rats were anesthetized, uterus and ovaries were collected for preimplantation embryo morphological analysis and counting of corpora lutea numbers, respectively.

Results: C. americana extract reduced the number of blastocysts in the C and D groups in relation to the untreated groups. DT dams presented no blastocyst collected on GD4, and CT and D rats presented a higher number of pre-embryos with morphological changes compared to the control group. The pre-embryos from CT, D and DT dams presented retarded development but no statistical difference was observed among the groups. In addition, the ratio of implanted pre-embryos by corpora lutea numbers in CT and DT rats was 15 and 72%, respectively, and higher compared to control groups.

Conclusion: Maternal diabetes or C. americana extract causes abortifacient effects confirmed by fewer pre-embryos in early pregnancy. C. americana extract previously caused pre-embryo fixation before implantation window, reinforcing the caution of indiscriminate use of medicinal plants without a medical prescription, especially during pregnancy.

#63 Curatella americana extract impacts on the embryo implantation of offspring from diabetic rats

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Introduction: The indiscriminate use of plant extracts to treat diseases and avoid the conception is widespread in several countries. Curatella americana L. is used as a menstrual cycle regulator and to prevent diabetes, but their effects are unclear in pregnancy.

Objective: To evaluate the effects of C. americana aqueous extract on the oestrous cycle and preimplantation embryos of adult offspring from diabetic rats. Methods: The first generation (Sprague-Dawley mother rats) was chemically induced to diabetes (D) or not (ND) and mated to obtain their female offspring (O). At adulthood, these offspring were distributed into four experimental groups (n = 6 animals/group): (1) female offspring from control mothers and received vehicle (OC) or (2) given plant extract (OC/T); (3) female offspring from diabetic mothers and received water (OD) or (4) given plant extract (OD/T). C. americana leaves aqueous extract was daily given by gavage. Vaginal smears were collected during consecutive ten days. Following, OC, OC/T, OD and OD/T rats were mated and on gestational day 4 (GD4) the rats were anesthetized and uterus was removed for preimplantation embryo morphological analysis.

Results: OD rats exhibited irregular oestral cycles, with fewer days in dioestrous phases and more days in estrous compared to OC. On GD4, the OD group presented higher percentage of anomalous pre-embryos and pre-embryo losses. After C. americana treatment, OD/T dams presented persistent days in metaestrus and absent dioestrus (follicular phase) compared to other groups. Plant extract caused greater number of corpora lutea in OD/T rats, stimulating ovulation. Regardless of maternal diabetes influence, the plant previously anticipated embryo implantation. In addition, the non-implanted pre-embryos collected on GD4 presented retarded development (as morulae stage) and abnormalities.

Conclusion: C. americana must be avoided, especially in early pregnancy, because it impairs reproductive cycles and compromises preimplantation embryo development.

#64 Low vitamin E levels have moderate effects in the expression of neurogenesis markers in brains of HDL-receptor SR-B1 KO mouse fetuses

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Vitamin E (VE) has been linked to brain development/function as an antioxidant protecting neurons and glia. VE is taken up via the HDL receptor Scavenger receptor class B member 1 (SR-B1), which is expressed in the blood-brain barrier and in extraembryonic tissues. Mouse E9.5 SR-B1-/- embryos have undetectable VE levels and high-incidence (1:2) of neural tube defects (NTD). Here, we compared VE content and expression of neurogenesis markers in brains of SR-B1-/- and WT embryos/fetuses. During neurogenesis, neural stem cells (NSC) proliferate and differentiate as they migrate from the ventricular to the cortical zone. Different transcription factors control this

process, such as Sox2 (in NSCs), Pax6 (in radial glial cells), Tbr2 (in intermediate neuronal progenitors or INPs) and DCX (in immature neurons). We used existing RNAseq data and compared the expression of markers of neurogenesis SR-B1-/- and WT littermates at E9.5. We also retrieved fetuses from SRB1+/- intercrosses at E16.5 and subjected their brains to HPLC to quantify VE, western blot (WB) using specific primary antibodies or RT-qPCR using specific primers. Another set of brains were fixed in PFA4%, snap-frozen in OCT, sectioned (20 μm) and subjected to immunofluorescence (IF) using specific antibodies. Our result showed reduced expression of neurogenic factors Sox2 and Pax6 ($p < 0.005$) in E9.5 embryos. In E16.5 fetuses, lower VE levels were detected in SR-B1-/- (n = 6) vs. WT (n = 6) brains (330.8 vs 1260 μg/μg protein, $p = 0.024$). Tbr2 levels were also lower in SR-B1-/- brains, as observed in WB (0.79 vs 1.07 RU in WTs, $p = 0.005$) and IF experiments. No differences were observed in Sox2, Pax6 or DCX levels. Future studies will determine the relevance of vitamin E deficiency and lower Tbr2 expression in SR-B1-/- fetal brains. Funding: FONDECYT #1180347 (DB) and Ph.D. fellowship ANID #21201204 (FS).

#65 Maternal severe diabetes damages fetal and placental development of the next generation: experimental study

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Background: Evidence indicates that maternal diabetes programs the fetus for diabetes at adulthood. Our hypothesis is that maternal severe diabetes causes alterations on the granddaughters in the embryofetal development period.

Objective: To evaluate the effect of maternal decompensated diabetes on fetal and placental development of the next generation of rats.

Methods: Diabetes was induced in adult life of female rats by beta-cytotoxic agent (STZ – intraperitoneal route – 40 mg/kg dose). The diabetic state was confirmed by blood glucose levels = 300 mg/dL. The nondiabetic (Control) rats and diabetic group were mated to obtain their offspring, which were classified according to the body weight as adequate (AGA), small (SGA) or large (LGA) weight for gestational weight. At adulthood, the offspring AGA from control dams (OCONT_AGA) and SGA from severe diabetic dams OSD_SGA were submitted to Oral Glucose Tolerance Test to estimate the total area under the curve (AUC) and for diabetes status confirmation. Next, these rats were mated. At the end of pregnancy, the rats were anesthetized and uterus were collected for fetal and placental weights. *T* student test or Fisher exact test were used. For statistical comparisons, a minimum confidence limit of 95% ($p < 0.05$) was considered.

Results: The OSD_SGA rats had higher AUC, lower fetal weight and placental efficiency compared to the OCONT_AGA group. In the group OSD_SGA, there was a lower percentage of newborns classified as AGA and higher percentage of SGA descendants.

Conclusion: The maternal inadequate intrauterine environment caused by decompensated diabetes negatively affected the growth of their female offspring (SGA) and impaired glucose homeostasis during pregnancy. The adult OSD_SGA presented lower placental efficiency, impairing the supply of nutrients to the fetuses, leading to an increase of grandnewborns classified as small for pregnancy age. These experimental findings show an inadequate maternal diabetic environment compromises fetal programming over other generations.

#66 Diabetes or protein restriction diet-induced fetal programming impair reproductive outcomes and fetal viability in rats

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Introduction: Diabetes mellitus and malnutrition negatively affects maternal environment, contributing to a damaged fetal programming. However, several inadequate maternal intrauterine environments on female reproductive outcomes to future generations are still unclear.

Objective: To evaluate the influence of maternal diabetes or protein restriction diet on the reproductive performance and fetal growth of adult offspring.

Methods: Maternal diabetes was induced by beta-cytotoxic drug (streptozotocin) at birth. The maternal malnutrition was caused by protein-restricted diet, which was prepared using only 6% of protein (standard food: 17% of protein) and given during pregnancy and lactation. Sprague Dawley adult rats were submitted to oral glucose tolerance test to confirm diabetes status. Next, the nondiabetic, diabetic and protein-restricted diet rats were distributed into four experimental groups (n = 8 animals/group): Female offspring from nondiabetic rat mother (Control – OC); Female offspring from mildly diabetic mothers (OMD); Female offspring from severely diabetic mothers (OSD), and Female offspring from mothers who received a protein-restricted diet (OPR). The rats were mated and, at the end of pregnancy, were anesthetized; their uterus and ovaries were collected for counting of corpora lutea, implantation and fetal numbers. The fetuses and placentas were weighed for placental efficiency analysis.

Results: OPR group had lower embryo implantation and alive fetus numbers, and lower litter weight compared to C, OMD and OSD groups; and a higher number of embryonic deaths than C and OSD groups. All groups presented lower fetal weight compared to the control group, and the OSD dams had lower weight of fetuses compared to OPR and OMD groups. OMD group showed greater placental weight than C, OPR and OSD rats.

Conclusion: The offspring from malnourished rats present an impaired process of embryo implantation and fetal development. The maternal diabetes compromises next generations confirmed by from placental efficiency, damaging fetal growth.

#67 Uncontrolled diabetes in the rat pregnancy impaired the glucose metabolism and reproductive outcomes of the next generation

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Introduction: Fetal programming can be caused by any disturbance during the gestational period, such as Diabetes mellitus. This syndrome disturbs glucose and insulin metabolism, leading to damaged fetal development and other repercussions at adulthood of these offspring.

Objective: To evaluate the effect of maternal decompensated diabetes on reproductive outcomes of the next generation of rats. Methods: Diabetes was induced in adult life of female rats by beta-cytotoxic agent. The diabetic state was confirmed by blood glucose levels = 300 mg/dL. The nondiabetic (Control) rats and diabetic group were mated to obtain their offspring, which were classified according to the body weight as adequate (AGA), small (SGA) or large (LGA) weight for gestational weight. At adulthood, the offspring AGA from control dams (OCONT_AGA) and SGA from severe diabetic dams OSD_SGA were submitted to Oral Glucose Tolerance Test for diabetes status confirmation. Next, these rats were mated. At the end of pregnancy, the rats were anesthetized and uterus were collected for counting of the numbers of alive fetuses, embryonic death, implantations and of corpora lutea, and rates of pre and postimplantation losses. *T* Student and Fisher's exact tests were used. For all statistical comparisons, $p < 0.05$ was considered as significant limit.

Results: The OSD_SGA rats coming presented higher blood glucose levels (mg/dL) in OGTT timepoints (fasting: 128.2 ± 9.96; 30 min = 155.09 ± 21.51; 60 min = 147.82 ± 11.1; 120 min = 109.27 ± 7.04) compared to OCONT_AGA timepoints (77.89 ± 7.27; 91.44 ± 24.28; 86.56 ± 21.74; 78.44 ± 8.97). The alive fetus, embryonic death, implantation and corpora lutea numbers, and embryo postimplantation losses had no difference between the groups. However, OSD_SGA rats presented higher embryo losses.

Conclusion: The severe diabetic state in pregnant rats interfere with fetal development, causing a lower birth weight on the newborns. Furthermore, these female pups showed an abnormal glucose metabolism, leading to damaged embryo development. More studies are needed to understand the mechanisms behind these impairments.

#68 Maternal intrauterine environment as a generator of changes in preimplantation embryos of rat generations

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Introduction: Pregestational hyperglycemia affects mothers and their offspring. The changes during pregnancy have been related to spread of the phenotype of diabetes across generations.

Objective: To evaluate and compare the morphological alterations in preimplantation embryos from severly and mildly diabetic rats and from offspring of mildly diabetic dams.

Methods: Mild and severe diabetes was induced in female Sprague Dawley rats by streptozotocin (beta cytotoxic drug) in different life ages. The non-diabetic females received the citrate buffer (vehicle). At adulthood, the rats were submitted to an oral glucose tolerance test (OGTT) for confirmation of mild or severe diabetes status. Following, the control (C), mildly diabetic (MD) and severely diabetic (SD) rats were mated to obtain preimplantation embryos on gestational day 4 (GD4). Other group of MD rats were also mated to obtain offspring (OMD). These OMD rats were mated to obtain preimplantation embryos on GD4. Pre-embryos were examined according to embryo numbers and morphology.

Results: The corpora lutea numbers showed no statistical difference among experimental groups. The SD and OMD rats had lower number of blastocysts collected on GD4 compared to C and MD dams. The SD rats presented 50% of preimplatantion embryo losses and OMD dams had 37% of losses compared to control and mild diabetic rats. OMD dams showed higher number of blastocysts with retarded development (as morulae or zygote) in compared to

other groups. This finding might impairs later implantation process. SD and MD had a higher number of pre-embryos morphological changes compared to C and OMD rats.

Conclusion: The hyperglycemic intrauterine environment directly caused morphological changes in the preimplantation embryo, but a more intense hyperglycemia in severely diabetic rats caused a higher number of abnormal pre-embryos that damaged their development. The intrauterine diabetes from last generation caused delay of development of the blastocyst, confirming the intergenerational effect of diabetes.

#69 Reproductive and systemic effects induced by coconut oil, lard and soybean oil in female mice

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Obesity leads to several health problems and also give rise to reproductive dysfunction. The aim of this current study was to determine the effects of coconut oil (CO), lard (LA) and soybean oil (SO) supplementation on reproductive cycle in female mice. Female Swiss mice (5–6 weeks old; 9 mice/group) were divided into the treatments group (control, coconut oil, lard and soybean oil) and the mice in each group were given 0.6 mL of the corresponding lipid source daily for 6 weeks. We assessed the changes in body weight, food consumption, metabolic tolerance (glucose and insulin tolerance). Vaginal smears were performed daily in all mice to track the reproductive cycle across the experimental groups. Our results showed a significant increase in body weight of all lipid supplemented groups when compared to the control ($P < 0.05$). Within the treated groups, final body weights of mice in the SO group were higher than those of the LA and CO groups. Metabolic tolerance analysis showed impaired glucose tolerance in the CO and SO groups when compared with the control. Estrous cycle analysis showed elongation of phases (for example, diestrus) in the SO group, skipping of phases (for example metestrus) in the SO and CO group, this revealed disruption in the reproductive cycle. These results show that excessive intake of soybean oil and coconut oil may cause increase body weight gain and metabolic changes which may likely have a negative impact on the hypothalamic-pituitary axis and affect reproduction, which in turn has effect on the estrous cycle and thereby causing reproductive dysfunction.

#71 The impact of intrauterine growth restriction on growth and livermorphology throughout the postnatal development of pigs

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Intrauterine growth restriction (IUGR) is a significant cause of fetal, neonatal and infant mortality and morbidity, often resulting from functional placental insufficiency. Considering that human studies may be difficult due to practical issues, IUGR animal models have been used to investigate maternal-fetal interactions. In this sense, the pig considered a potential model because exhibits IUGR naturally severe due to placental insufficiency. Some evidences suggest an association between IUGR and hepatic dysfunctions, as well metabolic syndrome in early life, but little is known about its long-term effects. In this sense, the objective of this research was to investigate the effect of IUGR on their growth and their hepatic parameters, evaluating morphology of the liver and blood levels of hepatic enzymes from newborn, weaned and adult piglets, as well as their biochemical status. For this, 324 piglets were divided into two groups: high weight (HW) and IUGR according to the birth weight. At these ages, body biometry was performed and 10 pairs of piglets (10 HW and 10 IUGR) were euthanized for the collection of blood samples and fragments of liver tissue for biochemical and histometric analysis, respectively. Biometric data indicates that IUGR

negatively affects their lifelong growth, and besides, low weight was associated with higher serum levels of glucose and cholesterol. Our results show that liver weight was regained in IUGR group at 150 days, even though IUGR animals remained lighter than their brothers. The histometric parameters and the blood levels of hepatic enzymes TGO and TGP were similar between the groups at all ages, which suggests that the IUGR liver is whether heterogeneously affected in its morphology or lighter at birth for having fewer cells, an asymmetric IUGR-like characteristic. We concluded that IUGR causes long-term physiological alterations in pigs, but the morphology of affected organs may be more adaptable.

#10067 Caffeine consumption during pregnancy and lactation impairs postnatal development and sexual maturation of female mice offspring

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Caffeine is the most widely consumed psychoactive substance in the world. even during pregnancy. Due to its ability to cross the placenta, it can accumulate in the fetus' and neonate's body. However, its postnatal effects on the offspring still remain unclear. This study was conducted to investigate the effects of maternal daily consumption of caffeine during pregnancy and/or lactation on postnatal development and sexual maturation attainment of female offspring. Forty adult female WT Swiss mice were randomly divided into four experimental groups: CC, received water throughout the experiment; TC, received caffeine only during pregnancy; CT, received caffeine only during lactation, and TT, received caffeine during both gestation and lactation. Caffeine was diluted in water (120 mg/kg/day for mice or 300 mg/day for human) and given through gavage, as well as water in the CC group. After birth, female puppies were weighed and had the crown-rump length determined. These measures were taken every five days until adulthood (60 days). Vaginal opening was monitored daily, starting 20 days after birth, and. Weaning performed at 30 days. Our results showed that caffeine consumption by the dams caused noticeable changes in biometrical characteristics throughout postnatal development, particularly in animals whose mothers consumed caffeine during lactation. Offspring from the TT group showed lower body weight and shorter crown-rump length from the neonatal period up to adulthood ($P < 0.05$). Furthermore, there was a notable delay in the timing of vaginal opening, mainly in groups CT and TT ($P < 0.05$). Thus, caffeine consumption during lactation, even in doses currently recommended by international organizations, may compromise postnatal development and affect female sexual maturation in female offspring.

#10068 The first evidence of progesterone's metabolite (4-pregnene) effects in human ovarian cancer cell lines

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Ovarian cancer is the fifth leading cause of cancer-related death among women and is the second most common type of gynecologic cancer diagnosed during pregnancy. It is known that ovarian cancer is detected in advanced stages of the disease. Although the incidence of ovarian cancer in pregnancy is low and the maternal-fetal prognosis is

generally favorable for the early stages of the tumor, management depends on the stage of the disease, the length of gestation, and the patient's wishes. Termination of pregnancy in the early stages is usually the main decision of patients who prioritize treatment or the desire to preserve reproductive capacity. Among the most important hormones for the maintenance of pregnancy, Progesterone and its metabolites present a sustained increase until the time of delivery. Currently, the role of progesterone in ovarian carcinogenesis is controversial. This hormone can be metabolized into the 4-pregnenes, 3a-di-hydroprogesterone and 20a-dihydroprogesterone derivatives. These steroids are active and have anti-tumor effects in breast cancer. Here, we evaluated the effect of 4-pregenenes derivates on cell proliferation (MTT), and tumor migration (wound assay) of two human ovarian cancer lines, IGROV-1 and SKOV-3. In both lines, 3a-di-hydroprogesterone and 20a-dihydro-progesterone significantly reduced proliferation of the ovarian cancer cell lines. Migration, a critical event in metastasis formation, was significantly stimulated by 20a-dihydro-progesterone on IGROV-1. This effect was concentration-dependent with a maximum of 298% for 10–11M ($p < 0.0001$). Meanwhile, 3a-di-hydroprogesterone did not present any significant differences with respect to the control in this line. SKOV-3 migration was not modified by these steroids. This research is a first step in understanding the effects of 4-pregnane derivatives on the biological behavior of these two cell lines derived from ovarian tumors. Understanding how these steroids work could have important future implications both in the treatment of cancer patients and in women's reproductive health.

#10069 Developmental Neurotoxicity of Arsenic: Cognitive and Motor Consequences of Early Infancy

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Brief Introduction: Arsenic is a known neurotoxic element. In utero, exposure to toxic metals substances can cause severe neurodevelopmental deficits in the developing fetus as well as infants. The evidences on impact of prenatal arsenic exposure on neurodevelopment at early infancy are scarcity from India.

Objectives: Cases of miscarriage, stillbirth, and children's cognitive and motor impairment due to metal toxicity continue to be on a rise in developing countries. We explored the prenatal arsenic levels at 3rd Trimesters of pregnancy and in cord blood and its effect on infant neurodevelopment at five months of age.

Methodology: We included 96 month-child pair, a prospective cohort study in Rajasthan, India. We used a separate linear regression model to evaluate the effect of arsenic exposure level with Infant neurodevelopment at five months of age. The Bayley Scales of Infant Development-3rd Edition was used to assess the Infant's neurodevelopment. Maternal and cord blood arsenic concentration was measured in whole blood using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Outcome: Separate linear regression models showed no association of arsenic exposure during late pregnancy (Median; 1.81 μg/dL (1.23, 2.39)) and birth (umbilical cord blood) (Median; 1.28 μg/dL (0.88, 1.39)) on infant neurodevelopment (Cognitive, Motor, Language and social-emotional development domain) at average age of five months.

Conclusion: Maternal blood showed higher arsenic levels than cord blood, suggesting an effective placental barrier for arsenic. No correlation between arsenic concentration in maternal and cord blood and child neurodevelopment at early infancy was found. Perhaps long-term follow-up may help to evaluate the cumulative impact of prenatal exposure to low arsenic levels on children's neurodevelopment.

-COVID-19, pathogens and reproductive health

#11 Zika induces inflammation in placenta and BDNF expression can modules fetal microcephaly

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In Brazil, an epidemic of Zika virus (ZIKV) infections was declared in 2015 that coincided with alarming reports of microcephaly in newborns associated with mother infection. Although the virus has placental tropism, changes in the tissue morphology and immunity of infected patients have not yet been elucidated. Here, we investigated the histopathological and ultrastructural changes along with the immunological profile and the BDNF expression in rare placental material. Tissues were obtained in the 2015–2016 Brazilian epidemic, of ten ZIKV-infected patients during pregnancy, five resulting in cases of fetal microcephaly and five non-microcephaly, compared to five non-infected control placentae. Viral antigens were only detected in samples from the ZIKV infected patients. Infected placentae presented histopathological severe damage, while the ultrastructural evaluation showed abnormal organelles, such as clusters of virus-like particles consistent with the ZIKV dimensions. Increased infiltration of $CD68^+$ and $TCD8^+$ cells, expression of MMPs, cytokines (IFN-γ and TNF-α) and other immunological mediators (RANTES/CCL5 and VEGFR-2) confirmed excessive inflammation and vascular permeability dysfunction. An evaluation of BDNF showed a decrease that could modulate neuronal damage in the developing fetus. The placental changes caused by ZIKV are not pathognomonic, however, the data provide evidence that this infection leads to severe placental injury.

#30 Prostatitis effect on male fertility

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Introduction: Chronic infections of the genitourinary tract are associated with altering male fertility and even promoting carcinogenic processes. Among the most frequent alterations in men and includes chronic prostatitis, whose effect on male fertility is still a controversial issue, so the objective of this work was to evaluate the effect of chronic prostatitis on fertility by comparing seminal quality.

Materials and methods: Eleven fertile volunteers and ten chronic prostatitis symptoms volunteers were evaluated semen quality using conventional functional parameters. In addition, nitric oxide and some pro-inflammatory

cytokines were quantified in semen and seminal plasma samples, the expression of the ROR-γT, FOX P3 and T BET genes in semen samples and the presence of DNA of microorganisms associated with prostatitis in urine and semen.

Results: No statistically significant differences were observed for conventional seminal parameters and functional measurement of cytokines, the expression of ROR-γT genes, FOX P3 and T BET, or measurements of PSA, antioxidant or nitrite concentration; however, chronic prostatitis is associated with sexual dysfunctions such as premature ejaculation.

Discussion: Chronic prostatitis is a common disease in young men that alters sexual and reproductive health and sometimes male fertility. Promoting male self-care should be a priority issue in public sexual health policies because men can present pain associated with prostatitis for long periods of time without receiving medical attention.

Conclusion: Although chronic prostatitis affects quality of life and sexual and reproductive health, it does not appear to affect male fertility.

Author contributions

The ISRH2021 Organizing Committee (GAA, PA, JSB, JAS, MLW) contributed equally to the organization of 2021 ISRH.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.