2021

medicina

BUENOS AIRES Vol. 81 Supl. III - 2021

MEDICINA

Volumen 81, Supl. III, Págs. 1- 260

medicina

BUENOS AIRES, VOL. 81 Supl. III - 2021

La Tapa
Médanos
Daniela Kantor

MEDICINA (Buenos Aires) - Revista bimestral – ISSN 1669-9106 (En línea)

Registro de la Propiedad Intelectual N° 02683675
Personería Jurídica N° C-7497
Publicación de la Fundación Revista Medicina (Buenos Aires) Propietario de la publicación: Fundación Revista Medicina
Queda hecho el depósito que establece la Ley 11723

Publicada con el apoyo del Ministerio de Ciencia, Tecnología e Innovación Productiva.
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Incluida en el Núcleo Básico de Revistas Científicas Argentinas del CONICET.

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Vol. 81, Supl. III, Noviembre 2021

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REUNIÓN DE SOCIEDADES DE BIOCIENCIAS 2021

LXVI REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

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17-20 de noviembre de 2021

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posed as a potential diagnostic indicator for different clinical conditions. Urine CfDNA detection would provide a tool for a less invasive prospective diagnosis. Limited information about urinary cfDNA detection is available, and cfDNA stability is a challenge due to urine variable pH. We optimized a reliable method for cfDNA isolation and detection from urine. Urine samples from 18 to 40-year-old women were centrifuged at high revolutions to remove cell debris. To assess whether urine acidity influences isolation efficiency, acidic (pH5) and neutralized (pH7) urine samples were assayed. cfDNA was isolated from 800 μL urine samples aliquots using QIAamp DNA Blood Mini Kit (QIAGEN) and eluted with 50 μL elution buffer. cfDNA concentration was measured by Nanodrop spectrophotometer. A real time quantitative PCR was performed using an optimized protocol for B-Actin amplification. Different cfDNA volumes (5 and 10 μL) and dilutions (1/2, 1/4 and 1/8) in a 20 μL final volume were assayed. Amplification products were analyzed by 1.5% agarose gel electrophoresis. cfDNA concentrations in neutralized and non-neutralized samples were 4.7 and 5.3 ng/μL, respectively. Specific B-Actin amplification product, in both neutralized and non-neutralized samples, was detected at Tm=80.1 °C; but at Tm=75.4 °C a non-specific amplification product was also detected. Sample neutralization prior to isolation considerably decreased non-specific amplification products. Furthermore, Cts values obtained demonstrated amplification progression in cfDNA successive dilutions assayed, with low standard deviation between duplicates (difference <0.5). We isolated and detected cfDNA from urine, and demonstrated the importance of performing sample neutralization prior to isolation. Although limited information about urine-cfDNA is available, it may be a promising biological biomarker.

545. (295) **THE OTHER SIDE OF COVID-19 PANDEMIC: EFFECTS ON FEMALE FERTILITY**

Yamila Herrero[1], Natalia Pascuali[1], Candela Velázquez[1], Gonzalo Oubiña[1], Vanesa Hauk[2], Ignacio de Zúñiga[3], Mariana Gómez Peña[3], Gustavo Martínez[4], Mariano Lavolpe[5], Florencia Veiga[6], Fernando Neuspiller[6], Dalhia Abramovich[1], Leopoldina Scotti[1,7]and Fernanda Parborell[1].

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SARS-CoV-2 invades the target cell by binding to angiotensin converting enzyme 2 (ACE-2). In the human ovary, ACE-2 is expressed in stromal and granulosa cells.
Our objective was to evaluate the effect of SARS-CoV-2 infection on female gonad.
FF (follicular fluid) from patients undergoing ART (n= 80; 21–41 years old; November 2020-April 2021) were divided in two groups: FF from control patients and FF from recovered COVID-19 patients (asymptomatic and with mild symptoms).
The levels of IgG antibodies against SARS-CoV-2, IL-1β, IL-10 and VEGF were measured in FF by ELISA.
Using a granulosa cell line (COV434) and an endothelial cell line (EA.hy926), we studied the effect of FF from control and recovered COVID-19 patients. The expression of StAR, ERα and ERβ, 3β-HSD, VEGF, ANGPTs (angiogenesis-related proteins) and γH2AX (DNA damage marker) was evaluated by WB. Proliferation was evaluated by a WST-1 assay. Endothelial cell migration was evaluated by a wound healing assay. We performed Student's t test or one-way ANOVA.
The results showed that 91.3% of post-COVID-19 FF was positive for IgG against SARS-CoV-2. Patients with higher levels of SARS-CoV-2 IgG showed a decrease in the number of retrieved oocytes ($p<0.05$). The levels of VEGF and IL-1β were lower ($p<0.05$) in post-COVID-19 FF, while IL-10 did not differ.
In COV434 cells with post-COVID-19 FF, the expression of StAR, Erβ and VEGF was decreased ($p<0.05$), while ERα and 3β-HSD did not change.
In EA.hy926 cells with post-COVID-19 FF, a decrease in cell migration was observed ($p<0.0001$) without changes in the expression of ANGPTs. Both cell types showed higher expression of γH2AX with post-COVID-19 FF ($p<0.05$). No differences were found in COV434 and EA.hy926 cell proliferation rates between the groups.
In conclusion, these results describe that SARS-CoV-2 infection alters the follicular microenvironment, damaging ovarian function, and affecting reproductive performance in recovered COVID-19 patients.
This project that involves the use of human samples from assisted fertilization techniques has been approved by the IByME Ethics Committee in 2020 (REGISTRATION CODE 2850, October 2020).
This project was carried out between January and June 2021.

546. (342) **COPPER CHELATION INHIBITS ANGIOGENESIS AND MODULATES THE OXIDATIVE IMBALANCE IN A MODEL OF ENDOMETRIOSIS**

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Endometriosis (EDT) is an estrogen-dependent disease that affects 5-15% of reproductive-aged women. It is characterized by the growth of endometrial-like tissue outside the uterine cavity and often causes chronic pelvic pain and subfertility. Currently, EDT has no cure, and there is an unmet need for new treatment options. Angiogenesis is essential for the growth of endometriotic implants because it ensures an adequate supply of oxygen and nutrients and the removal of waste products. Elevated copper (Cu) levels have been linked to EDT. Cu is required by many enzymes, some involved in the antioxidant system. In cancer, this metal promotes angiogenesis, tumor progression, and oxidative stress. Therefore, our objective was to evaluate the effect of Cu chelation with ammonium tetrathiomolybdate (TM) on angiogenesis and oxidative stress in endometriotic-like lesions. Sixteen female C57BL/6 mice were divided into two experimental groups: EDT and EDT+TM. The EDT induction was performed by autologous uterine tissue transplantation to the intestinal mesentery. The EDT+TM group received 0.30 mg of TM/day in their drinking water for two weeks from the postoperative 15th day. Bodyweight and hematocrit were periodically monitored. Endometriotic-like lesions were collected one month after the pathology was induced to analyze the expression of angiogenic markers (RTqPCR), the presence of endothelial cells (immunofluorescence), and oxidative stress (spectrophotometric methods). Treatment with TM induced anti-angiogenic effects by decreasing the number of blood vessels ($p<0.001$), the mRNA expression of *Fgf2* and *Pdgfb* ($p<0.05$), and the presence of endothelial cells ($p<0.001$). Besides, it decreased antioxidant activity (SOD and CAT, $p<0.05$) and increased lipid peroxidation (TBARS, $p<0.05$). In conclusion, TM acts as an effective anti-angiogenic agent and modulates the oxidative imbalance in EDT. These observations support the study of TM as a possible non-hormonal treatment for EDT.

547. (350) **ORAL INFECTION AND ADVERSE PREGNANCY OUTCOME: *PORPHYROMONAS GINGIVALIS* OUTER MEMBRANE VESICLES ENTER TROPHOBLAST CELLS**