NUTRIVISA

ORIGINAL ARTICLE

https://doi.org/10.52521/nutrivisa.v12i1.15667

Effects of continuous ethinylestradiol and drospirenone administration on body mass and mammary gland in female mice fed a standard or high-fat diet

Efeitos da administração contínua de etinilestradiol e drospirenona sobre a massa corporal e glândula mamária em camundongos fêmeas alimentadas com dieta normo e hiperlipídica

Gésily de Souza AGUIAR[1] Janaína de Oliveira CHAVES (In Memorian)[2]
Israelle Netto FREITAS[1] Kênia Moreno de OLIVEIRA[3] Ana Beatriz Barbosa da SILVA[2]
Stefanny Calixto da SILVA[2] Rosane Aparecida RIBEIRO[4] Helene Nara Henriques BLANC[2]*

[1]Universidade Federal do Rio de Janeiro – NUPEM UFRJ Macaé – RJ – Brasil

[2]Universidade Federal do Rio de Janeiro – Centro Multidisciplinar UFRJ Macaé – RJ – Brasil

[3]Universidade Estadual de Campinas – Campinas – SP – Brasil

[4]Universidade Estadual de Ponta Grossa – PR – Brasil

*Corresponding author: helenenara@hotmail.com

ABSTRACT

Combined oral contraception (COC) is used by millions of women worldwide, but some studies suggest it may increase the risk of breast cancer. Overweight and obesity have reached epidemic levels and are also risk factors for mammary gland neoplasms. This study evaluated the effects of a COC containing ethinylestradiol (EE2) and drospirenone (DRSP) on reproductive organs, particularly the histopathology of inguinal mammary glands in reproductively active female mice fed a standard diet (SD) or high-fat diet (HFD). Adult Swiss female mice received either SD or HFD and were treated daily by gavage with distilled water [control (CTL)-SD and CTL-HFD] or 0.6 µg EE2 and 60 µg DRSP (COC-SD and COC-HFD) for 65 days. COC treatment disrupted the estrous cycle, with vaginal smears showing fewer squamous cornified cells but more leukocytes, deep cells, and mucus, characteristic of metestrus and proestrus. At the end of the experiment, COC-SD females showed body weight (BW), inguinal white adipose tissue (WAT) mass, and uterus and ovary weights similar to CTL-SD. HFD increased BW and WAT mass without altering uterus and ovary weights. COC reduced HFD-induced BW and WAT gain but increased uterus weight in COC-HFD females. Morphological analysis of mammary glands revealed no pathological signs or structural changes in acini, ducts, or lumens due to COC or diet. Notably, COC-HFD females had smaller white adipocytes than CTL-HFD, while beige adipocyte levels remained unchanged across groups. These findings suggest that EE2 and DRSP may regulate HFD-induced obesity without adversely affecting mammary gland morphology.

Keywords: mammary gland; obesity; oral contraceptives.

RESUMO

Contraceptivos orais combinados (COC) são utilizados por milhões de mulheres, mas estudos sugerem que podem aumentar o risco de câncer de mama. Sobrepeso e obesidade, também fatores de risco para neoplasias mamárias, atingiram proporções epidêmicas. Este estudo avaliou os efeitos de um COC com etinilestradiol (EE2) e drospirenona (DRSP) sobre órgãos reprodutivos, especialmente as características histopatológicas das glândulas mamárias inguinais em camundongos fêmeas adultas sexualmente ativas alimentadas com dieta padrão (SD) ou hiperlipídica (HFD). As fêmeas Swiss receberam SD ou HFD e, por 65 dias, gavagem diária de água destilada [controle (CTL)-SD e CTL-HFD] ou 0,6 µg EE2 + 60 µg DRSP (COC-SD e COC-HFD). O COC alterou o ciclo estral, com esfregaços vaginais indicando redução de células escamosas e aumento de leucócitos, células profundas e muco, típicos de metaestro e proestro. No fim do experimento, COC-SD apresentou peso corporal (PC), massa de tecido adiposo branco (TAB) inguinal e pesos uterino e ovariano semelhantes aos CTL-SD. A HFD aumentou o PC e o TAB, sem afetar útero e ovários. O COC atenuou esses aumentos na HFD, mas elevou o peso uterino em COC-HFD. A análise morfológica das glândulas mamárias não mostrou alterações patológicas ou estruturais em ácinos, ductos ou luz. COC-HFD apresentou adipócitos brancos menores que CTL-HFD, enquanto a quantidade de adipócitos bege permaneceu inalterada. Esses achados sugerem que EE2 e DRSP podem regular a obesidade induzida por dieta rica em gordura sem afetar negativamente a morfologia das glândulas mamárias.

Palavras-chave: glândula mamária; obesidade; contraceptivos orais.

Cite this article as

AGUIAR , G. de S.; CHAVES (in memorian), J. de O.; FREITAS, I. N.; OLIVEIRA, K. M. de; SILVA, A. B. B. da; SILVA, S. C. da; RIBEIRO , R. A.; NARA HENRIQUES BLANC, H. Effects of continuous ethinylestradiol and drospirenone administration on body mass and mammary gland in female mice fed a standard or high-fat diet. Nutrivisa Revista de Nutrição e Vigilância em Saúde, Fortaleza, v. 12, n. 1, p. e15667, 2025. DOI: 10.52521/nutrivisa.v12i1.15667. Disponível em: https://revistas.uece.br/index.php/nutrivisa/article/view/15667.

INTRODUCTION

Oral contraceptives are the most prevalent contraceptive method among Brazilian women aged 15 to 49 years (UNITED NATIONS, 2024). These contraceptives can be composed of both an estrogen and a progestin, known as combined oral contraceptives (COCs), or containing only a progestin. Natural and synthetic estrogens may be used in COC formulations, with ethinylestradiol (EE2) being the most utilized estrogen. Progestins are classified into generations based on their time of market introduction. Drospirenone (DRSP) is a fourth-generation progestin derived from spironolactone that exhibits antiandrogenic and antimineralocorticoid properties that beside of its antigonadotropic and antiovulatory actions, DRSP may contribute to reduced water retention and mitigation of hirsutism (Fuhrmann *et al.*, 1996; Krattenmacher, 2000; Muhn *et al.*, 1995; Rosenbaum *et al.*, 2000).

Evidence suggests that the use of COCs may reduce the risk of certain gynecological cancers; however, it has also been associated with an increased risk of some types of breast cancer in young and premenopausal women (Barańska, 2022; Fitzpatrick *et al.*, 2023; Graafland, Abbott e Accordino, 2020). Despite this, research on the effects of different COC formulations on mammary health remains limited, and the impact of COCs containing EE2 and DRSP on the mammary gland is largely unexplored. Previous studies have shown that DRSP, when administered together with estradiol in ovariectomized mice, increases side-branch formation and mammary gland proliferation at doses higher than those used in COCs. In perimenopausal women, hormone therapy with estradiol and DRSP for 12 months has been associated with increased breast density (Kiran *et al.*, 2011). Similarly, in postmenopausal women, such hormone therapy has been linked to increased breast density due to the proliferation of breast epithelial cells (Hirschberg *et al.*, 2019).

Overweight and obesity have reached epidemic proportions worldwide (World Obesity Federation, 2024). These conditions increase the risk of various cancers, including breast cancer (Cao *et al.*, 2024; Hillers-Ziemer e Arendt, 2020; Nguyen *et al.*, 2023). This increased risk may be attributed to alterations in mammary gland tissue proliferation and apoptosis rates, inflammation, dysregulation of adipokines, and extracellular matrix remodeling (Hillers-Ziemer e Arendt, 2020). However, the combined impact of obesity and COC use on mammary gland health and breast cancer development remains largely unexplored. One study reported that, in postmenopausal women, obesity and hormonal contraceptive use tended to increase the percentage of aggressive breast cancers, although the findings were not statistically significant (Agustin e Barokah, 2019). This study aims to evaluate the effects of a combined oral contraception (COC) composed of ethinylestradiol (EE2) and drospirenone (DRSP) on reproductive organs, with a particular focus on the histopathological features of the inguinal mammary glands in reproductively active female mice fed either with a standard diet (SD) or a high-fat diet (HFD).

MATERIALS AND METHODS

Ethical considerations in animal experimentation

The study was conducted in accordance with Brazilian legislation on procedures for the scientific use of animals of the Phylum Chordata, Subphylum Vertebrata (excluding humans; Federal Law 11.794 of October 8, 2008) and the current normative resolutions of the National Council for the Control of Animal Experimentation (CONCEA) of Ministry of Science, Technology, and Innovation of Brazil. It was approved by the Ethics Committee on Animal Use of the Federal University of Rio de Janeiro under protocol number MAC039.

Experimental groups

Adult female Swiss mice (aged 80 to 100 days) were collective cages housed in the Rodent Experimental Facility (DBPio, Multidisciplinary Center, UFRJ-Macaé) under controlled conditions of temperature (21 ± 2°C), humidity, and light cycles (lights on: 7 am to 7 pm). For 65 days, the female mice were fed on a standard diet (SD; Nuvilab CR1, Quimtia, Colombo, PR, BRA) or high-fat die (HFD) and daily received a gavage of 200 µL of distilled water (control (CTL)-SD and CTL-HFD groups) containing or not 0.6 µg of EE2 and 60 µg of DRSP (COC-SD and COC-HFD groups,

respectively). Throughout the experimental period, the female mice had free access to their respective diets and filtered water.

The contraceptive used was a COC commercial tablet containing 30 µg of EE2 and 3 mg of DRSP (EMS Pharma, Hortolândia, SP, BRA). The conversion of this dose used in humans to the corresponding dose in mice was performed using allometric calculation (Freitas e Carregaro, 2013) to approximate the daily concentration ingested by a woman who weighs 60 kg. The HFD was prepared using SD, which provided 3.8 kcal/g, of which 9% was derived from fat (Nuvilab CR1, Quimtia, Colombo, PR, BRA). This chow was ground into a powder and mixed with lard (Aurora, Chapecó, SC, BRA) to increase the lipid content. Additionally, protein content was adjusted by adding purified casein (Synth, Diadema, SP, BRA), and essential fatty acid levels were balanced with soybean oil (Bunge Alimentos, São Paulo, SP, BRA). The HFD provided 5.6 kcal/g, with 60% of the energy coming from lipids.

Estrous cycle analysis

Vaginal cytology was collected biweekly and on the day of euthanasia using a spatula inserted into the vaginal opening, obtaining a smear by rotating it 360°. The sample was spread on pre-identified glass slides, which were immediately fixed in 95% alcohol and later stained using the Papanicolaou method. Vaginal cytology analysis is based on the response of vaginal epithelium to hormonal stimulation. This evaluation determines the estrous cycle phase, which in mice comprises four phases: estrus, metestrus, diestrus, and proestrus. Each phase lasts an average of one day, and a complete estrous cycle occurs every four days (Raimondi *et al.*, 2024).

Euthanasia and collection of inguinal mammary glands and uterus weights collection

Euthanasia of CTL and COC groups began on the 65th day of experimental period. As the administration of COC can interfere in the secretion of gonadotropins and ovarian steroids in COC groups, the CTL-SD and CTL-HFD females were euthanized only at the meta-estrus phase of the estrous cycle phase, since the plasma concentrations of gonadotrophins, estrogen, and progesterone can be near to that is induced by oral contraception (Smith, Freeman e Neill, 1975). To confirm this, vaginal smears were collected from CTL-SD and CTL-HFD females on the day of the euthanasia using 0.9% saline solution and observed under light microscopy (Olympus CX31) (Oliveira *et al.* 2019). At the day of euthanasia, CTL and COC female mice were weighed, anesthetized with isoflurane, and then submitted to decapitation. The skin of the inguinal region was cut and the right and left inguinal white adipose tissues (WAT) containing the mammary glands, were dissected and weighed. Subsequently a laparotomy was performed to access the uterus which was excised and weighed.

Histopathological and Histomorphometric Analysis of the Mammary Gland

The inguinal WAT containing the mammary glands of CTL and COC females that fed on a SD or a HFD was fixed in 10% Carson's formalin for 48 h. The tissues were then dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Sections of 5 µm in thick were obtained and stained with hematoxylin and eosin (H&E). A general descriptive analysis was performed by observing the histological sections stained with H&E under an optical microscope (Novel BM 2100, China) to examine the structure of mammary ducts and acini, adipose and connective tissue, as well as the presence of pathological features such as hyperplasia, dysplasia, neoplasia, atrophy, apoptosis, necrosis, mitosis, calcifications, lipofuscin accumulation, cellular vacuolization, and secretion.

For histomorphometric analysis of the mammary glands, one section per female mouse, and five fields per section stained with H&E were photographed with a digital camera (Tucsen USB 2.0 H series, China) coupled to a light microscope (Novel BM 2100, China) at 200 x magnification. The histomorphometric analysis of the mammary gland also included the quantification of percentage areas of mammary components. To determine the percentage areas in each mammary gland image, a grid of points was applied to the image using the "Grip plugin" of the software ImageJ (https://imagej.net/ij/). The duct-acini, WAT-muscle, lumen structures overlapped by the points in the grip were manually counted and registered. The number of duct-acini, WAT-muscle, lumen structures per field analyzed were multiplied by 100 and divided by the

number of points in whole gland. Afterward, the total percentage of mammary gland, WAT/muscle areas was calculated by summing the duct-acini or WAT/muscle areas from the five analyzed fields and dividing the total by five (Pompei *et al.*, 2005).

For adiposity analysis, five random fields of the inguinal WAT containing the mammary gland section from each female mouse were photographed at 400x magnification in a light microscope (Novel BM 2100, China) coupled to a light microscope (Novel BM 2100, China). The horizontal and vertical diameters of 12 adipocytes distributed in each field were manually measured using the "line" tool of the ImageJ software (https://imagej.net/ij/).

Histomorphometric Analysis of the Mammary Gland by Whole Mount

After euthanasia, the right inguinal WAT containing mammary glands were stretched on silanized slides and pressed. They were then fixed in 10% Carson's formalin for 24 h, and then dehydrated in 100% alcohol for 30 min, rehydrated in descending alcohol solutions and stained with toluidine blue for 12h followed by dehydratation and clearing with xylene for 24h. Subsequently five randomly fields per mammary gland were photographed at 200x magnification in a light microscope (Novel BM 2100, China) coupled to a light microscope (Novel BM 2100, China). For each mammary gland field, a grid of points was applied with the aid of the ImageJ software (https://imagej.net/ij/). The total number of points relative to all structures of the mammary gland covered by the grip was counted, multiplied by 100, divided by the total number of points in the grip, and then divided by the number of analyzed fields to obtain a mean. Also, the number of points covering the branches or alveoli of the mammary gland was manually counted and recorded, and the mean total of these structures across the five analyzed fields was determined. (Tolg, Cowman e Turley, 2018).

Gene Expression

Fragments of inguinal WAT containing the mammary gland were subjected to total RNA extraction using Trizol® (ThermoFisher, Waltham, MA, USA). Samples of 1 µg of RNA were used for reverse transcription to synthesize complementary DNA (cDNA) using random primers, 100 mM DTT, 10 mM dNTP mix, and 200U of SuperScript II enzyme. Real-time polymerase chain reactions were performed in a final volume of 10 µL, containing 12.5 ng of cDNA, 3 pM of primers, and Sybr Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA), and detected using the 7500 Real-Time PCR system (Applied Biosystems, Waltham, MA, USA). Gene expression was determined using the 2-ΔΔCT method, and the relative mRNA expression of each target gene in this study was normalized to the expression of the glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene. The genes examined play a crucial role in understanding the physiological changes that occur in the mammary gland following hormone exposure in the context of obesity. Their expression provides insight into processes such as hormonal signaling and energy metabolism (Adrβ1, Adrβ3), regulation of apoptosis (Bcl-2), and mechanisms of cell proliferation and differentiation (Igf-2, Cyclin D1). Notably, the dysregulation of certain genes has been frequently linked to the development of mammary tumors (Casimiro *et al.*, 2013; Conway *et al.*, 2023; Derossi *et al.*, 2003; Valentine *et al.*, 2022). The genes analyzed and the sequences of the primers used are shown in Table 1.

Statistical analysis

Data are presented as means ± SEM. The results were evaluated for normal distribution using the Shapiro-Wilk test. Subsequently the variances were compared using parametric (one-way ANOVA followed by Tukey's post-test) or non-parametric analysis (Kruskal-Wallis followed by Dunn's). The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

General reproductive and biometric features of control (CTL) and combined oral contraception (COC) females that fed on a standard diet (SD) or a high-fat diet (HFD)

The effectiveness of hormonal treatment with contraceptive hormones can be evaluated through vaginal smears, as sex hormones act on the vaginal epithelium, among other target tissues, and COC administration alters the typical cycling pattern. In pigtail macaques

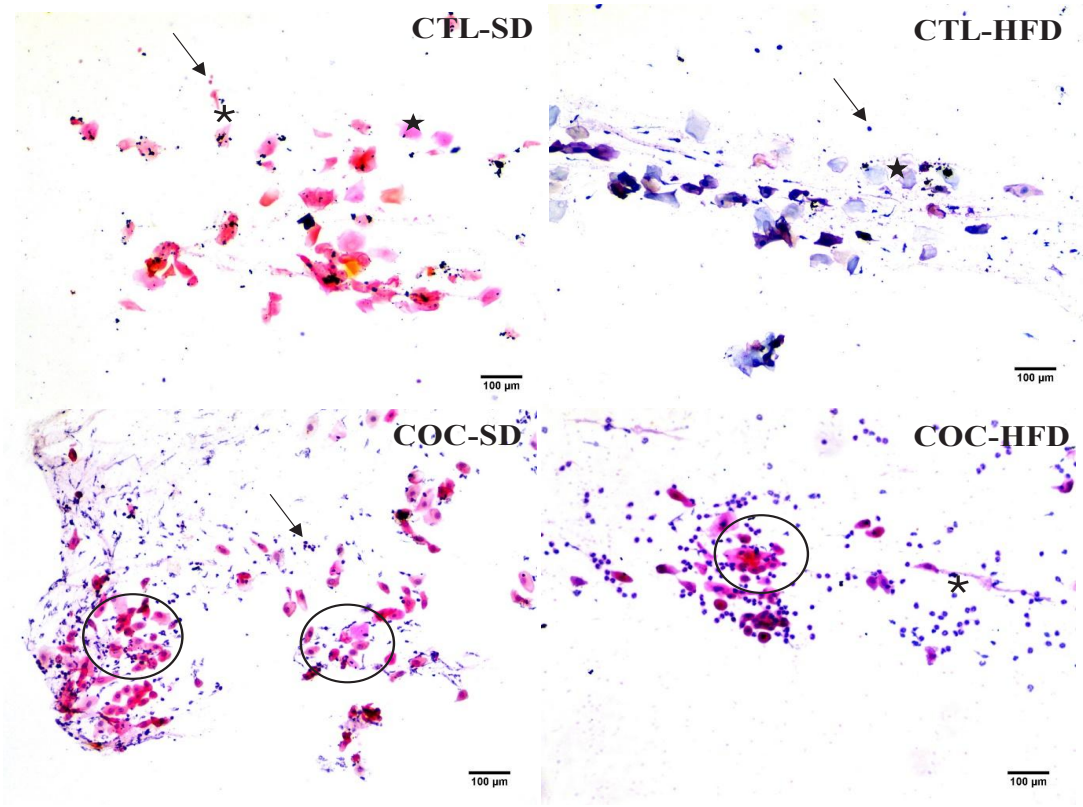
the administration of EE2 and levonorgestrel gradually led to the loss of the non-nucleated layer in the vaginal epithelium (Dietz Ostergaard *et al.*, 2015). In accordance, the administration of estradiol and progesterone demonstrated to increase the number of leukocytes, deep cells and mucus in vaginal smears of rat at reproductive age and in ovariectomized rats (Henriques, 2013). The same estrous cycle effect was observed in Wistar female rats treated with combined injectable contraceptive composed of estradiol valerate and norethisterone enanthate (NERY *et al.*, 2021). Thus, throughout the experimental period, the estrous cycle was monitored to assess the previously mentioned effect of COC. In agreement with these literature data and with whose was observed in a study that administered EE2 and DRPS for 35 days to female mice (Gouveia *et al.*, 2020), COC treatment in both COC-SD and COC-HFD groups stopped cycling normally, and the vaginal cytology plateaued, showing characteristics of the metestrus and proestrus phases, as shown in Figure 1. Is possible to observe that vaginal smears of COC-SD and COC-HFD females exhibited little cornified squamous cells, higher leukocytes and mucus (characteristic of metestrus), and increased number of deep cells slightly grouped (characteristic of proestrus). On the other hand, vaginal smears collected from CTL-SD and CTL-HFD female mice exhibited all four phases of the estrous cycle. As shown in Figure 1, the metaestrus phase recorded on the day of euthanasia for CTL-SD and CTL-HFD females is characterized by cornified squamous cells, leukocytes, and mucus. Therefore, the changes in the estrous cycle observed in the COC groups indicated an expected COC effect on vagina. Additionally, the results demonstrated that the type of diet consumed did not interfere with this effect.

As observed in a previous study that treated female mice with EE2 and DRSP for 35 days (Oliveira *et al.*, 2019), here, the prolongation of COC treatment for 65 days also did not alter BW in COC-SD females (Fig.: 2A). In addition, COC administration did not change the weight of inguinal WAT containing the mammary glands (Fig.: 2B), and the weight of uterus (Fig.: 2C) or ovary (Fig.: 2D) of COC-SD females, when compared to CTL-SD. It is important to mention that another effect of COC administration on reproductive organs is the increase in uterus weight (Dietz Ostergaard *et al.*, 2015; Dixon *et al.*, 2024; Oliveira *et al.*, 2019), but here, despite of a trend toward to increase in uterus weight of COC-SD (Fig.: 2C), it was not significant different from CTL-SD ($p = 0.14$). On the other hand, in accordance with previous studies (Figueiredo *et al.*, 2020; Oliveira *et al.*, 2020), HFD consumption increased BW and the weight of the inguinal WAT in CTL-HFD, in comparison with CTL-SD females ($p < 0.05$ and $p < 0.001$; Fig.: 2A and 2B). As recently reported by our research group (Chaves *et al.*, 2025), here we observed that COC administration combined with HFD intake attenuated HFD-induced increases in BW and in the weight of inguinal WAT (Fig.: 2A and 2B). In addition, COC-HFD females displayed higher uterus weight, than that observed in CTL-HFD ($p < 0.0001$; Fig.: 2C). This effect may be linked to the hormonal effect of the COC in uterus, which manifest in part due to estrogenic actions of the synthetic hormone that composes the COC (CLEUREN *et al.*, 2010). No differences in ovary weight were observed among COC-HFD and CTL-HFD females (Fig.: 2D).

Notably, a previous study reported that EE2 and levonorgestrel administration to C57Bl/6 female mice attenuated the obesogenic effects of an HFD on BW and adiposity but also caused liver injury (Fuller *et al.*, 2022). Literature provides limited information on the effects of COC administration on obesity development. The sex hormones used in COCs may influence metabolism; for instance, estradiol administration has been shown to increase energy expenditure in female mice fed an HFD (Al-Qahtani *et al.*, 2017). Additionally, DRSP administration to female mice has been reported to attenuate HFD-induced increases in body adiposity (Armani *et al.*, 2014). Thus, it is possible that both EE2 and DRSP in the COC used in this study exerted metabolic effects that mitigated the obesogenic impact of the HFD. However, it is important to emphasize that our results do not support the use of EE2 and DRSP as a strategy for body weight reduction in obesity. Obesity is a known risk factor for insulin resistance, and EE2 and DRSP administration in female mice has also been identified as a potential contributor to this condition, as demonstrated by Oliveira *et al.* (Oliveira *et al.*, 2019). This highlights the necessity for further investigations into the effects

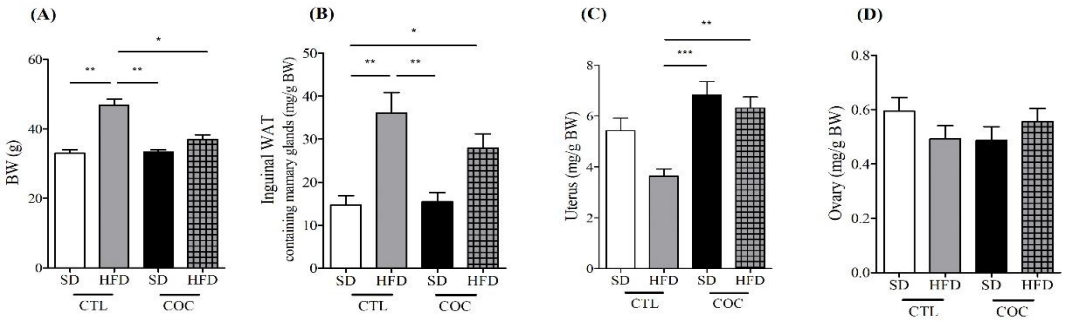
https://doi.org/10.52521/nutrivisa.v12i1.15667

Figure 1 – Representative images of Papanicolaou-stained vaginal smears collected on the day of euthanasia from control (CTL) and combined oral contraceptive (COC) treated female mice fed with a standard diet (SD) or high-fat diet (HFD).



In the CTL-SD and CTL-HFD groups cytology is consistent with metestrus, characterized by the presence of mucus (sinalized by *), leukocytes (sinalized by ↑), and cornified squamous cells (sinalized by ★). In the COC-SD and COC-HFD groups, hormonal stimulation by the combined oral contraceptive altered vaginal cytology, showing clusters of deep epithelial cells (sinalized by circles), combined with leukocytes (↑) and mucus (*). Scale bars = 100 µm.

Figure 2 – Body weight (BW), weight of inguinal white adipose tissue (WAT) containing mammary glands, uterus, and ovary of control (CTL) and combined oral contraceptive (COC) treated female mice fed with a standard diet (SD) or high-fat diet (HFD).



Data are means ± SEM of BW (A), and weights of inguinal WAT (B), uterus (C) and ovary (D) of CTL-SD (n = 17), CTL-HFD (n = 14), COC-SD (n = 10) and COC-HFD female mice (n = 19). Lines over the bars indicate statistical differences among the groups for the gene indicated. * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$. Data were analyzed using one-way ANOVA followed by Tukey's post-test, except for data in D, which were compared using the Kruskal-Wallis test followed by Dunn's post-test.

of EE2 and DRSP on intermediary metabolism and whether their metabolic impact is accompanied by impairments in overall body homeostasis.

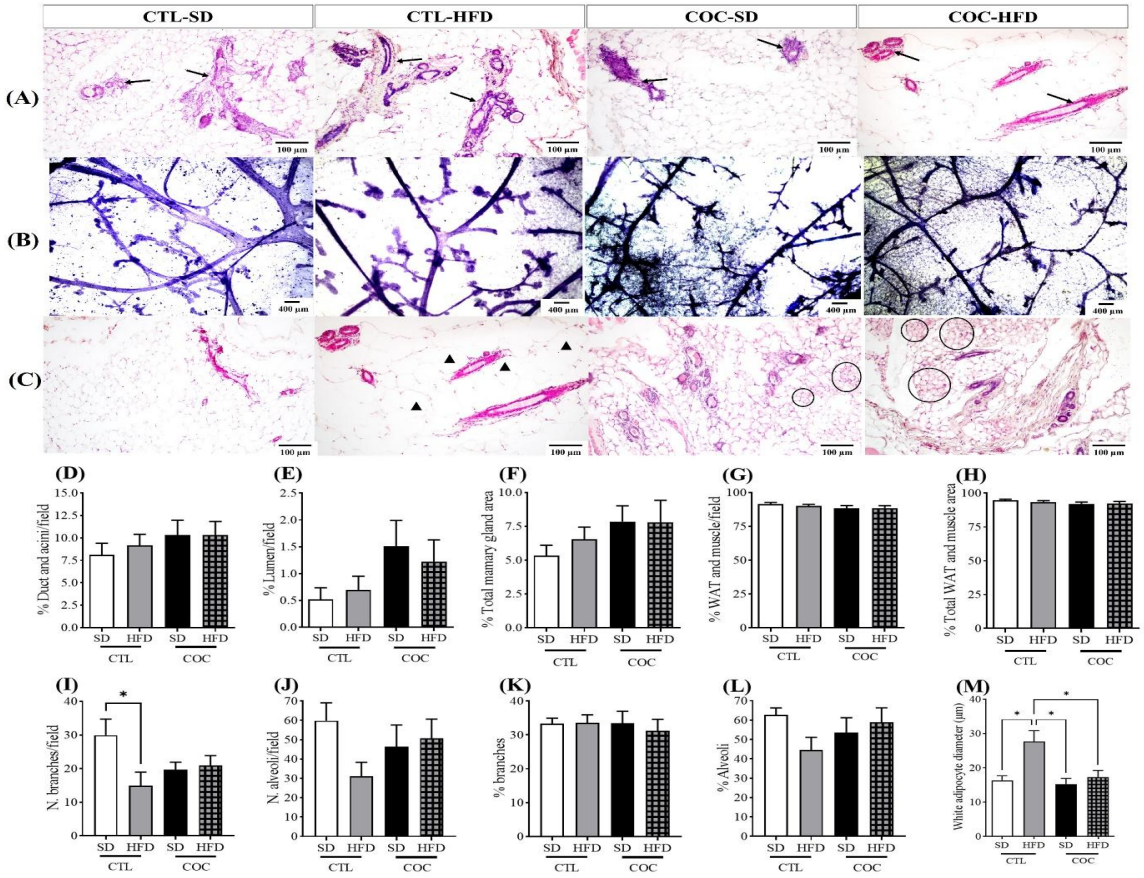
Mammary glands and inguinal adipose tissue morphology and molecular evaluation of control (CTL) and combined oral contraception (COC) females that fed on a standard diet (SD) or a high-fat diet (HFD).

The use of contraceptives has been related to increasing the risk to mammary gland neoplasias (Fitzpatrick *et al.*, 2023). In fact, estrogens and progestins may regulate mammary gland cellular proliferation and differentiation (Pompei *et al.*, 2005), however the literature has limited information about the effects of COC on morphology of this gland. In a study with rats treated with a combined injectable hormonal contraceptive, mammary hyperplasia was observed (Henriques, 2013), which is associated with an increased risk of developing breast cancer (Smolarek *et al.*, 2013) . Another studyusing 1 mg estradiol and 2 mg DRSP in perimenopausal women for 12 months has been linked to the increased breast density in mammography (Kiran *et al.*, 2011). Regarding DRSP effects, a previous study demonstrated that the subcutaneous administration of 100 ng/kg of estradiol with DRSP, whose doses vary from 0.8 to 180 mg/kg, in C57Bl/6 female mice dose dependently regulates the proliferation and structure of the mammary glands (Otto *et al.*, 2008). Thus, here the effect of EE2 and DRSP administration to female mice in a dose near of those used by women can be observed in Figure 3. In the qualitative histopathological analysis was observed that CTL and COC groups, independently of the diet ingested, displayed similar morphological characteristics of the mammary glands, exhibiting acini and ducts with slight luminal dilation and a small amount of secretion, without lipid droplets. Ducts and acini presented a cuboidal epithelium with one to two cell layers, featuring eosinophilic and non-vacuolated cytoplasm. No pathological alterations such as hyperplasia, dysplasia, neoplasia, atrophy, apoptosis, necrosis, mitosis, calcifications, lipofuscin accumulation, or cellular vacuolization were observed among CTL and COC females that fed on a SD or an HFD (Fig.: 3A).

Additionally, COC treatment did not change the percentage of the duct and acini (Fig.: 3D) and lumen (Fig.: 3E) areas, or the total percentage of mammary gland (Fig.: 3F), or fat-muscle areas (Fig.: 3G and 3H) in the inguinal WAT of COC-SD and COC-HFD females, when compared to CTL-SD and CTL-HFD groups, respectively. In agreement with these histopathological and histomorphometric analysis, whole mount histology revealed similar morphological features of the mammary glands of CTL and COC females that fed on a SD or an HFD, presenting ducts and acini with no histopathological findings (Fig.: 3B). COC treatment did not change the N. of branches (Fig.: 3I) and alveoli per field (Fig.: 3J), or the percentage of branches and alveoli (Fig.: 3K and 3L) between the COC-SD and CTL-SD females. HFD intake led to a significant reduction only in the N. of branches/field in CTL-HFD group ($p < 0.05$; Fig.: 3I), despite total branches in the mammary glands of these females was like CTL-SD (Fig.: 3K). COC treatment with HFD intake did not change the N. or percentage of branches and alveoli in mammary glands of COC-HFD, when compared to CTL-HFD (Fig.: 3B, 3I-3L). Therefore, these histopathological findings indicates that the COC composed for EE2 and DRSP when administered at a dose similar of those used by women, at least for 65 days, did not change the morphology of mammary glands in female mice, independently of the diet ingested.

Additionally, to the mammary gland tissue analysis, we performed an evaluation of the structure of the adipose tissue present in inguinal WAT, as can be seen in Figures 3C and 3M. COC treatment did not alter the size of white adipocytes of COC-SD females, when compared to CTL-SD (Fig.: 3C and 3M). In accordance with previous studies (Gao, Ma e Liu, 2015; Kubota *et al.*, 1999), HFD intake led to white adipocyte hypertrophy in CTL-HFD ($p < 0.05$; Fig.: 3C and 3M). COC treatment attenuated HFD-induced white adipocyte hypertrophy in inguinal WAT of COC-HFD females, since this group showed similar white adipocyte diameter of that observed for COC-SD females (Fig.: 3C and 3M). Recently we demonstrated that EE2 and DRSP administration for 65 days attenuated HFD-induced increase in adiposity, an effect possible linked to a higher thermogenic action in brown adipose tissue (Chaves *et al.*, 2025). In accordance, previously it was demonstrated that treatment with DRSP can lead to browning of the inguinal WAT (Armani *et al.*, 2014). Here, we investigated the area

Figure 3 – Histopathological analysis of the inguinal WAT containing mammary glands of control (CTL) and combined oral contraceptive (COC) treated female mice fed with a standard diet (SD) or high-fat diet (HFD).

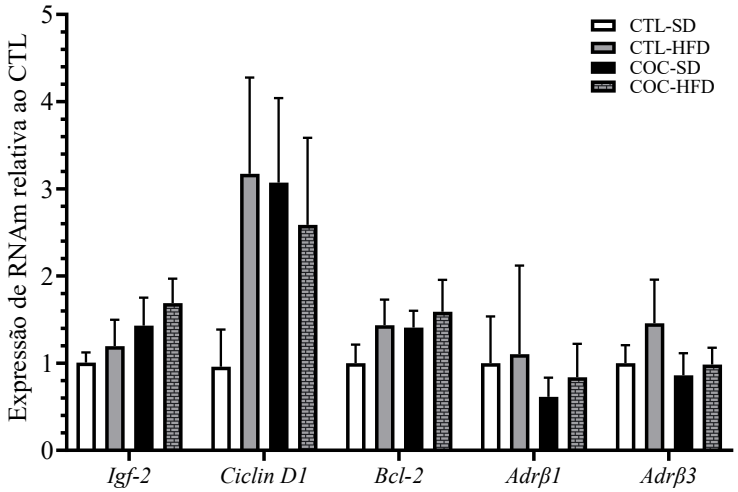


Representative histological sections stained with H&E or whole mounting images from the inguinal WAT containing the mammary glands of CTL and COC female mice that fed on a SD or an HFD. (A) arrows (↑) = presence of ducts and acini of the mammary glands stained with H&E. Scale bars = 100 µm. (B) branches and alveoli of the mammary glands submitted to whole mounting and stained with toluidine blue. Scale bars = 400 µm. (C) circle (o) = groups of beige adipocytes in inguinal WAT stained with H&E; triangle (▲) = hypertrophic white adipocytes in inguinal WAT of CTL-HFD females. Data are means ± SEM of the percentage of duct and acini (D), lumen (E), total mammary gland (F), fat and muscle (G) and total WAT and muscle areas (H), and of N. of branches (I) and alveoli per field analyzed (J), and of the percentage of branches (K) and alveoli of the mammary gland (L), and diameter of white adipocytes from inguinal WAT of CTL-SD (n = 17), CTL-HFD (n = 14), COC-SD (n = 10) and COC-HFD female mice (n = 19). Lines over the bars indicate statistical differences among the groups for the gene indicated. * $p < 0.05$. Data was analyzed using Kruskal-Wallis test followed by Dunn's post-test.

occupied by beige adipocytes in this adipose tissue depot and observed the presence of these cells in only 3 CTL-SD, 2 CTL-HFD, 4 COC-SD, and 3 COC-HFD females. Furthermore, the total area occupied by beige adipocytes in inguinal WAT containing mammary glands showed a tendency to vary among groups but was statistically similar: CTL-SD = 1032.0 ± 622.0, CTL-HFD = 75.6 ± 50.7, COC-SD = 920.9 ± 481.3 and COC-HFD = 848.4 ± 587.6 µm. This suggests that browning appears to be an intrinsic feature of mammary tissue that was not altered by COC or diet treatments in our study.

As sex hormones regulates various signaling pathways in mammary glands and adipose tissue metabolism (Arendt e Kuperwasser, 2015; Wawrzkiewicz-Jałowiecka, Lalik e Soveral, 2021), here we also investigated whether at molecular levels of some important genes involved in cellular proliferation in mammary tissues, as Igf-2, Ciclin D1 and Bcl-2 (Alimkhodjaeva *et al.*, 2023; Bates *et al.*, 1995), and in WAT lipolysis, were modified by COC treatment (figure 4). Despite the mRNA amounts of Igf-2, Ciclin D1, Bcl-2, showed a trend to vary among experimental groups, no statistically significant differences were

Figure 4 – Expression of genes involved in mammary gland trophism and lipid metabolism in the inguinal WAT containing mammary glands of control (CTL) and combined oral contraceptive (COC) treated female mice fed with a standard diet (SD) or high-fat diet (HFD).



Data are means ± SEM of the relative mRNA expression of *Igf-2*, *Ciclin D1*, *Bcl-2*, *Adrβ1* and *Adrβ3* in the inguinal WAT containing the mammary glands of CTL-SD (n = 6), CTL-HFD (n = 6), COC-SD (n = 6) and COC-HFD female mice (n = 6).

observed among COC and CTL groups. This result is consistent with our histopathological findings, which did not show mammary alterations related to cell proliferation and apoptosis. The cyclin D1, IGF-2, and bcl-2 genes play significant roles in mammary gland development and the pathology of breast cancer. These genes play a crucial role in mammary gland proliferation and apoptosis; therefore, they are linked to the tumorigenesis process (Boutinaud *et al.*, 2004; Casimiro *et al.*, 2013; Conway *et al.*, 2023; Derossi *et al.*, 2003; Kotsifaki *et al.*, 2024; Lee, Tocheny e Shaw, 2022; Liu *et al.*, 2022; Menezes, Oliveira e Barreto, 2021; Tiwari e Kaur, 2025). In our study, the absence of changes in the expression of these genes demonstrates the safety of this contraceptive in the obesity model. However, studies using this protocol for a longer period would be necessary to confirm this effect. Notably, a previous study demonstrated that 6 mg/kg DRSP administration to female C57Bl/6 mice increased Adrβ3 mRNA levels in subcutaneous WAT (Armani *et al.*, 2014). However, here, Adrβ1 and Adrβ3 genes exhibited a similar expression profile among CTL and COC females that fed on a SD or an HFD. Thus, further investigations are necessary to demonstrate how EE2 and DRSP alone and in combination may regulate lipid metabolism in subcutaneous WAT at obesogenic conditions.

Considering the high prevalence of hormonal contraceptive use in Brazil and the progressive increase in obesity rates among women, it is essential to investigate the effects of these agents in altered metabolic contexts. Obesity is associated with a chronic inflammatory state and changes in the metabolism of sex steroids, leading to elevated circulating levels of estrogen and adipokines—factors that promote cellular proliferation and are implicated in breast carcinogenesis, particularly in postmenopausal women (Iyengar *et al.*, 2016; Lauby-Secretan *et al.*, 2016). Simultaneously, epidemiological evidence indicates that current use of hormonal contraceptives, particularly combined formulations and those containing only progestins, is associated with a modest but significant increase in breast cancer risk (Mørch *et al.*, 2017; Skovlund *et al.*, 2016) (Mørch *et al.*, 2017; Skovlund *et al.*, 2018). Given the convergence of these factors (obesity and the use of exogenous hormones), both independently linked to increased breast cancer risk, future studies must evaluate their combined effects in an integrated manner, especially using experimental models. Such an approach may support safer and more individualized

regarding contraceptive counseling for women who are overweight or obese.

CONCLUSION

In summary, continuous administration of a COC composed of EE2 and DRSP in female mice at reproductive age attenuated HFD-induced increases in BW and inguinal WAT. However, this effect was not associated with changes in the number of a more metabolic active adipocytes, as beige adipocytes in the inguinal fat depot, or alterations in Adrβ1 and Adrβ3 gene expressions, which encode key receptors involved in adipocyte lipolysis. The absence of histopathological, histomorphometric, and molecular changes in the mammary glands of COC-SD and COC-HFD females suggests that EE2 and DRSP, at the doses and duration used in this study, may be considered safe in this experimental model.

REFERENCES

AGUSTIN, S.A.; BAROKAH, L. Correlation between Obesity and Contraceptive Method on Estrogen and Progesterone Receptors and Human Epidermal Growth Factor Receptor–2 Expression among Breast Cancer Patients in Dr. Moewardi Hospital, Surakarta. Indonesian Journal of Medicine, v.4, n.3, p.259–265, 2019. doi: 10.26911/theijmed.2019.4.3.204

ALIMKHODJAEVA, L.T.; NISHANOV, D.A.; BOZAROVA, L.M.; NORBEKOVA, M.K.H. Immunohistochemical Aspects of the Expression of Markers of Cell Cycle Regulation, Proliferation and Apoptosis (p53, Ki-67, bcl-2, cyclin D1) in Breast Neoplasia. Research In Cancer and Tumor, v.11, n.1, p.1–5, 2023. doi:10.5923/j.rct.20231101.01

AL-QAHTANI, S.M.; BRYZGALOVA, G.; VALLADOLID-ACEBES, I.; KORACH-ANDRÉ, M.; DAHLMAN-WRIGHT, K.; EFENDIĆ, S.; BERGGREN, P.O.; PORTWOOD, N. 17β-Estradiol suppresses visceral adipogenesis and activates brown adipose tissue-specific gene expression. Hormone Molecular Biology and Clinical Investigation, v.29, n.1, p.13–26, 2017. doi: 10.1515/hmbci-2016-0031

ARENDT, L.M.; KUPERWASSER, C. Form and Function: how Estrogen and Progesterone Regulate the Mammary Epithelial Hierarchy. Journal of Mammary Gland Biology and Neoplasia, v.20, n.1–2, p.9–25, 2015. doi: 10.1007/s10911-015-9337-0

ARMANI, A.; CINTI, F; MARZOLLA, V.; MORGAN, J.; CRANSTON, G.A.; ANTELMI, A.; CARPINELLI, G.; CANESE, R.; PAGOTTO, U.; QUARTA, C.; MALORNI, W.; MATARRESE, P.; MARCONI, M.; FABBRI, A.; ROSANO, G.; CINTI, S.; YOUNG, M.J.; CAPRIO, M. Mineralocorticoid receptor antagonism induces browning of white adipose tissue through impairment of autophagy and prevents adipocyte dysfunction in high- fat-diet- fed mice. The FASEB Journal, v.28, n.8, p.3745–3757, 2014. doi: 10.1096/fj.13-245415

BARAŃSKA, A. Oral Contraceptive Use and Assessment of Breast Cancer Risk among Premenopausal Women via Molecular Characteristics: Systematic Review with Meta-Analysis. International Journal of Environmental Research and Public Health, v.19, n.22, p.15363, 2022. doi: 10.3390/ijerph192215363

BATES, P.; FISHER, R.; WARD, A.; RICHARDSON, L.; HILL, D.; GRAHAM, C. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). British Journal of Cancer, v.72, n.5, p.1189–1193, 1995. doi: 10002E1038/bjc.1995.484

BOUTINAUD, M.; SHAND, J.; PARK, M.; PHILLIPS, K.; BEATTIE, J.; FLINT, D.; ALLAN, G. A quantitative RT-PCR study of the mRNA expression profile of the IGF axis during mammary gland development. Journal of Molecular Endocrinology, v.33, n.1, p.195–207, 2004. doi: 10.1038/bjc.1995.484

CAO, J.; LI, J.; ZHANG, Z.; QIN, G.; PANG, Y.; WU, M.; GU, K.; XU, H. Interaction between body mass index and family history of cancer on the risk of female breast cancer. Scientific Reports, v.14, n.1, p.4927, 2024. doi: 10.1038/s41598-024-54762-x

CASIMIRO, M.C.; WANG, C.; LI, Z.; SANTE, G. DI; WILLMART, N.E.; ADDYA, S.; CHEN, L.; LIU, Y.; LISANTI, M.P.; PESTELL, R.G. Cyclin D1 Determines Estrogen Signaling in the Mammary

Epithelial Hierarchy. Journal of Mammary Gland Biology and Neoplasia, v.20, n.1–2, p.9–25, 2015. doi: 10.1007/s10911-015-9337-0
ARMANI, A.; CINTI, F; MARZOLLA, V.; MORGAN, J.; CRANSTON, G.A.; ANTELMI, A.; CARPINELLI, G.; CANESE, R.; PAGOTTO, U.; QUARTA, C.; MALORNI, W.; MATARRESE, P.; MARCONI, M.; FABBRI, A.; ROSANO, G.; CINTI, S.; YOUNG, M.J.; CAPRIO, M. Mineralocorticoid receptor antagonism induces browning of white adipose tissue through impairment of autophagy and prevents adipocyte dysfunction in high-fat-diet-fed mice. The FASEB Journal, v.28, n.8, p.3745–3757, 2014. doi: 10.1096/fj.13-245415
BARAŃSKA, A. Oral Contraceptive Use and Assessment of Breast Cancer Risk among Premenopausal Women via Molecular Characteristics: Systematic Review with Meta-Analysis. International Journal of Environmental Research and Public Health, v.19, n.22, p.15363, 2022. doi: 10.3390/ijerph192215363
BATES, P.; FISHER, R.; WARD, A.; RICHARDSON, L.; HILL, D.; GRAHAM, C. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). British Journal of Cancer, v.72, n.5, p.1189–1193, 1995. doi: 10002E1038/bjc.1995.484
BOUTINAUD, M.; SHAND, J.; PARK, M.; PHILLIPS, K.; BEATTIE, J.; FLINT, D.; ALLAN, G. A quantitative RT-PCR study of the mRNA expression profile of the IGF axis during mammary gland development. Journal of Molecular Endocrinology, v.33, n.1, p.195–207, 2004. doi: 10.1038/bjc.1995.484
CAO, J.; LI, J.; ZHANG, Z.; QIN, G.; PANG, Y.; WU, M.; GU, K.; XU, H. Interaction between body mass index and family history of cancer on the risk of female breast cancer. Scientific Reports, v.14, n.1, p.4927, 2024. doi: 10.1038/s41598-024-54762-x
CASIMIRO, M.C.; WANG, C.; LI, Z.; SANTE, G. DI; WILLMART, N.E.; ADDYA, S.; CHEN, L.; LIU, Y.; LISANTI, M.P.; PESTELL, R.G. Cyclin D1 Determines Estrogen Signaling in the Mammary Gland In Vivo. Molecular Endocrinology, v.27, n.9, p.1415–1428, 2013. doi: 10.1210/me.2013-1065
CLEUREN, A.C.A.; LINDEN, I.K. VAN DER; VISSER, Y.P. DE; WAGENAAR, G.T. M.; REITSMA, P.H.; VLIJMEN, B.J.M. VAN. 17α-Ethinylestradiol rapidly alters transcript levels of murine coagulation genes via estrogen receptor α. Journal of Thrombosis and Haemostasis, v.8, n.8, p.1838–1846, 2010. doi: 10.1111/j.1538-7836.2010.03930.x
CONWAY, J.R.W.; DINÇ, D.D.; FOLLAIN, G.; PAAVOLAINEN, O.; KAIVOLA, J.; BOSTRÖM, P.; HARTIALA, P.; PEUHU, E.; IVASKA, J. IGFBP2 secretion by mammary adipocytes limits breast cancer invasion. Science Advances, v.9, n.28, 2023. doi: 10.1126/sciadv.adg1840
DEROSSI, D.R.; ITO, K.; COUTO FILHO, J.D.; BACCHI, C.E. Avaliação da expressão da proteína bcl-2 no carcinoma de mama: estudo em punção aspirativa por agulha fina; correlação com grau histológico em espécimes cirúrgicos correspondentes. Jornal Brasileiro de Patologia e Medicina Laboratorial, v39, n. 3, 2003. doi: 10.1590/S1676-24442003000300010
DIETZ OSTERGAARD, S.; BUTLER, K.; RITTER, J. M.; JOHNSON, R.; SANDERS, J.; POWELL, N.; LATHROP, G.; ZAKI, S. R.; MCNICHOLL, J.M.; KERSH, E.N. A combined oral contraceptive affects mucosal SHIV susceptibility factors in a pigtail macaque (Macaca nemestrina) model. Journal of Medical Primatology, v.44, n.2, p.97–107, 2015. doi: 10.1111/jmp.12157
DIXON, A.; ROWAN, E.G.; YACKLEY, A.N.; HINES, E.P. PFAS Modulate Osmotic Signaling Independent of Gravimetric Changes in the Rat Uterus. Toxics, v.12, n.3, p.170, 2024. doi: 10.3390/toxics12030170
FIGUEIREDO, L.S.; OLIVEIRA, K.M.; FREITAS, I.N.; SILVA, J.A.; SILVA, J.N.; FAVERO-SANTOS, B.C.; BONFLEUR, M.L.; CARNEIRO, E.M.; RIBEIRO, R.A. Bisphenol-A exposure worsens hepatic steatosis in ovariectomized mice fed on a high-fat diet: Role of endoplasmic reticulum stress and fibrogenic pathways. Life Sciences, v.256, p.118012, 2020. doi: 10.1016/j.lfs.2020.118012
FITZPATRICK, D.; PIRIE, K.; REEVES, G.; GREEN, J.; BERAL, V. Combined and progestagen-only hormonal contraceptives and breast cancer risk: A UK nested case–control study and meta-analysis. PLOS Medicine, v.20, n.3, p.e1004188, 2023. doi:

10.1371/journal.pmed.1004188
FREITAS, G.C.; CARREGARO, A.B. Aplicabilidade da extrapolação alométrica em protocolos terapêuticos para animais selvagens. Ciência Rural, v.43, n.2, p.297–304, 2013. doi: 10.1590/S0103-84782013000200017
FUHRMANN, U.; KRATTENMACHER, R.; SLATER, E.P.; FRITZEMEIER, K.H. The novel progestin drospirenone and its natural counterpart progesterone: Biochemical profile and antiandrogenic potential. Contraception, v.54, n.4, p.243–251, 1996. doi: 10.1016/s0010-7824(96)00195-3
FULLER, K.N.Z.; MCCOIN, C.S.; STIERWALT, H.; ALLEN, J.; GANDHI, S.; PERRY, C.G.R.; JAMBAL, P.; SHANKAR, K.; THYFAULT, J.P. Oral combined contraceptives induce liver mitochondrial reactive oxygen species and whole-body metabolic adaptations in female mice. The Journal of Physiology, v.600, n.24, p.5215–5245, 2022. doi: 10.1113/JP283733
GAO, M.; MA, Y.; LIU, D. High-Fat Diet-Induced Adiposity, Adipose Inflammation, Hepatic Steatosis and Hyperinsulinemia in Outbred CD-1 Mice. PLOS ONE, v.10, n.3, p.e0119784, 2015. doi: 10.1371/journal.pone.0119784
GOUVEIA, T.V.C.; AGUIAR, G.S.; CHAVES, J.O.; NASCIMENTO, D.S.C.; OLIVEIRA, C.A.R.; RIBEIRO, R.A.; LATINI, J.T.P.; BLANC, H.N.H. Efeito do uso ininterrupto de contraceptivo oral combinado na vagina de camundongos. Em: Tecnologia e Inovação para o Cuidar em Enfermagem. Atena Editora, p.99–109. 2020. doi: 10.22533/at.ed.94820261010
GRAAFLAND, L.; ABBOTT, M.; ACCORDINO, M. Breast Cancer Risk Related to Combined Oral Contraceptive Use. The Journal for Nurse Practitioners, v.16, n.2, p.116–120, 2020. doi: 10.1016/j.nurpra.2019.11.018
HENRIQUES, H.N. Efeito do uso contínuo de hormônios esteroides sexuais na mama de ratas Wistar. [Tese de Doutorado]. Niterói: Universidade Federal Fluminense, 2013.
HILLERS-ZIEMER, L.E.; ARENDT, L.M. Weighing the Risk: effects of Obesity on the Mammary Gland and Breast Cancer Risk. Journal of Mammary Gland Biology and Neoplasia, v.25, n.2, p.115–131, 2020. doi: 10.1007/s10911-020-09452-5
HIRSCHBERG, A.L.; TANI, E.; BRISMAR, K.; LUNDSTRÖM, E. Effects of drospirenone and norethisterone acetate combined with estradiol on mammographic density and proliferation of breast epithelial cells—A prospective randomized trial. Maturitas, v.126, p.18–24, 2019. doi: 10.1016/j.maturitas.2019.04.205
IYENGAR, N.M.; GUCALP, A.; DANNENBERG, A.J.; HUDIS, C.A. Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. Journal of Clinical Oncology, v.34, n.35, p.4270–4276, 2016. doi: 10.1200/JCO.2016.67.4283
KIRAN, H.; TOK, A.; YÜKSEL, M.; ARIKAN, D.C.; EKERBICER, H.C. Estradiol plus drospirenone therapy increases mammographic breast density in perimenopausal women. European Journal of Obstetrics & Gynecology and Reproductive Biology, v.159, n.2, p.384–387, 2011. doi: 10.1016/j.ejogrb.2011.09.023
KOTSIFAKI, A.; MAROULAKI, S.; KARALEXIS, E.; STATHAKI, M.; ARMAKOLAS, A. Decoding the Role of Insulin-like Growth Factor 1 and Its Isoforms in Breast Cancer. International Journal of Molecular Sciences, v.25, n.17, p.9302, 2024. doi: 10.3390/ijms25179302
KRATTENMACHER, R. Drospirenone: pharmacology and pharmacokinetics of a unique progestogen. Contraception, v.62, n.1, p.29–38, 2000. doi: 10.1016/s0010-7824(00)00133-5
KUBOTA, N.; TERAUCHI, Y.; MIKI, H.; TAMEMOTO, H.; YAMAUCHI, T.; KOMEDA, K.; SATOH, S.; NAKANO, R.; ISHII, C.; SUGIYAMA, T.; ETO, K.; TSUBAMOTO, Y.; OKUNO, A.; MURAKAMI, K.; SEKIHARA, H.; HASEGAWA, G.; NAITO, M.; TOYOSHIMA, Y.; TANAKA, S.; SHIOTA, K.; KITAMURA, T.; FUJITA, T.; EZAKI, O.; AIZAWA, S.; NAGAI, R.; TOBE1, K.; KIMURA, S.; KADOWAKI, T. PPARγ Mediates High-Fat Diet–Induced Adipocyte Hypertrophy and Insulin Resistance. Molecular Cell, v.4, n.4, p.597–609, 1999. doi: 10.1016/s1097-2765(00)80210-5
LAUBY-SECRETAN, B.; SCOCCIANTI, C.; LOOMIS, D.; GROSSE, Y.; BIANCHINI, F.; STRAIF, K. Body Fatness and Cancer — Viewpoint of the

IARC Working Group. New England Journal of Medicine, v.375, n.8, p.794–798, 2016. doi: 10.1056/NEJMsr1606602

LEE, J.S.; TOCHENY, C.E.; SHAW, L.M. The Insulin-like Growth Factor Signaling Pathway in Breast Cancer: An Elusive Therapeutic Target. Life, v.12, n.12, p.1992, 2022. doi: 10.3390/life12121992

LIU, N.Q.; CAO, W.H.; WANG, X.; CHEN, J.; NIE, J. Cyclin genes as potential novel prognostic biomarkers and therapeutic targets in breast cancer. Oncology Letters, v.24, n.4, p.374, 2022.

MENEZES, C.A.; OLIVEIRA, V.S.; BARRETO, R.F. Estudo da correlação entre obesidade e câncer de mama no período pré e pós-menopausa / Study of the correlation between obesity and breast cancer in the pre and post-menopause period. Brazilian Journal of Health Review, v.4, n.1, p.1487–1501, 2021. doi: 10.3892/ol.2022.13494

MØRCH, L.S.; SKOVLUND, C.W.; HANNAFORD, P.C.; IVERSEN, L.; FIELDING, S.; LIDEGAARD, Ø. Contemporary Hormonal Contraception and the Risk of Breast Cancer. New England Journal of Medicine, v.377, n.23, p.2228–2239, 2017. doi: 10.1056/NEJMoa1700732

MUHN, P.; KRATTENMACHER, R.; BEIER, S.; ELGER, W.; SCHILLINGER, E. Drospirenone: A novel progestogen with antimineralocorticoid and antiandrogenic activity. Contraception, v.51, n.2, p.99–110, 1995. doi: 10.1016/0010-7824(94)00015-o

NERY, L.C.D.E.S.; BRAZ, L.C.S.; FERREIRA, L.L.D.M.; VIEIRA, F.P.; SILVA, L.L.; BLANC, H.N.H.; RAIMUNDO, J.M. A combined injectable contraceptive improves plasma redox status and does not induce vascular changes in female rats. Anais da Academia Brasileira de Ciências, v.93, n.3, 2021. doi: 10.1590/0001-3765202120201924

NGUYEN, H.L.; GEUKENS, T.; MAETENS, M.; APARICIO, S.; BASSEZ, A.; BORG, A.; BROCK, J.; BROEKS, A.; CALDAS, C.; CARDOSO, F.; SCHEPPER, M.; DELORENZI, M.; DRUKKER, C.A.; GLAS, A.M.; GREEN, A.R.; ISNALDI, E.; EYFJÖRÐ, J.; KHOUT, H.; KNAPPSKOG, S.; KRISHNAMURTHY, S.; LAKHANI, S.R.; LANGEROD, A.; MARTENS, J.W.M.; MCCART REED, A.E.; MURPHY, L.; NAULAERTS, S.; NIK-ZAINAL, S.; NEVELSTEEN, I.; NEVEN, P.; PICCART, M.; PONCET, C.; PUNIE, K.; PURDIE, C.; RAKHA, E.A.; RICHARDSON, A.; RUTGERS, E.; VINCENT-SALOMON, A.; SIMPSON, P.T.; SCHMIDT, M.K.; SOTIRIOU, C.; SPAN, P.N.; TAN, K.T.B.; THOMPSON, A.; TOMMASI, S.; BAELEN, K.V.; VIJVER, M.V.; LAERE, S.V.; VAN'T VEER, L.; VIALE, G.; VIARI, A.; VOS, H.; WITTEVEEN, A.T.; WILDIERS, H.; FLORIS,G.; GARG, A.D.; SMEETS, A. LAMBRECHTS, D.; BIGANZOLI, E.; RICHARD, F.; DESMEDT, C. Obesity-associated changes in molecular biology of primary breast cancer. Nature Communications, v.14, n.1, p.4418, 2023. doi: 10.1038/s41467-023-39996-z

OLIVEIRA, C.A.R.; ARAUJO, T.R.; AGUIAR, G.S.; DA SILVA JUNIOR, J.A.; VETTORAZZI, J.F.; FREITAS, I.N.; OLIVEIRA, K.M.; BOSCHERO, A.C.; BONFLEUR, M.L.; CLARKE, J.R.; HENRIQUES, H.N.; RIBEIRO, R.A. Combined oral contraceptive in female mice causes hyperinsulinemia due to β-cell hypersecretion and reduction in insulin clearance. Journal of Steroid Biochemistry and Molecular Biology, v.190, p.54–63, 2019. doi: 10.1016/j.jsbmb.2019.03.018

OLIVEIRA, K.M.; FIGUEIREDO, L.S.; ARAUJO, T.R.; FREITAS, I.N.; SILVA, J.N.; BOSCHERO, A.C.; RIBEIRO, R.A. Prolonged bisphenol-A exposure decreases endocrine pancreatic proliferation in response to obesogenic diet in ovariectomized mice. Steroids, v.160, p.108658, 2020. doi: 10.1016/j.steroids.2020.108658

OTTO, C.; FUCHS, I.; ALTMANN, H.; KLEWER, M.; WALTER, A.; PRELLE, K.; VONK, R.; FRITZEMEIER, K.H. Comparative Analysis of the Uterine and Mammary Gland Effects of Drospirenone and Medroxyprogesterone Acetate. Endocrinology, v.149, n.8, p.3952–3959, 2008. doi: 10.1210/en.2007-1612

POMPEI, L.M.; CARVALHO, F.M.; ORTIZ, S.C.B.C.; MOTTA, M.C.; CRUZ, R.J.; MELO, N.R. Morphometric evaluation of effects of two sex steroids on mammary gland of female rats. Maturitas, v.51, n.4, p.370–379, 2005. doi: 10.1016/j.maturitas.2004.09.007

RAIMONDI, G.M.; ENG, A.K.; KENNY, M.P.; BRITTING, M.A.; OSTROFF, L.E. Track-by-Day: A standardized approach to estrous cycle monitoring

in biobehavioral research. Behavioural Brain Research, v.461, p.114860, 2024. doi: 10.1016/j.bbr.2024.114860
ROSENBAUM, P.; SCHMIDT, W.; HELMERHORST, F.M.; WUTTKE, W.; ROSSMANITH, W.; FREUNDL, F.; THOMAS, K.; GRILLO, M.; WOLF, A.; HEITHECKER, R. Inhibition of ovulation by a novel progestogen (drospirenone) alone or in combination with ethinylestradiol. The European Journal of Contraception & Reproductive Health Care, v.5, n.1, p.16–24, 2000. doi: 10.1080/13625180008500376
SKOVLUND, C.W.; MØRCH, L.S.; KESSING, L.V.; LIDEGAARD, Ø. Association of Hormonal Contraception With Depression. JAMA Psychiatry, v.73, n.11, p.1154, 2016. doi: 10.1001/jamapsychiatry.2016.2387
SMITH, M.S.; FREEMAN, M.E.; NEILL, J.D. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology, v.96, n.1, p.219–226, 1975. doi: 10.1210/endo-96-1-219
SMOLAREK, A.K.; SO, J.Y.; THOMAS, P.E.; LEE, H.J.; PAUL, S.; DOMBROWSKI, A.; WANG, C.X.; SAW, C.L.L.; KHOR, T.O.; KONG, A.N.T.; REUHL, K.; LEE, M.J.; YANG, C.S.; SUH, N. Dietary tocopherols inhibit cell proliferation, regulate expression of ERα, PPARγ, and Nrf2, and decrease serum inflammatory markers during the development of mammary hyperplasia. Molecular Carcinogenesis, v.52, n.7, p.514–525, 2013. doi: 10.1002/mc.21886
TIWARI, S.; KAUR, K. Clinico-pathological association of BCL2 in invasive breast carcinoma: A study from tertiary care health centre in Northern India. Indian Journal of Pathology and Microbiology, v.68, n.2, p.324–327, 2025. doi: 10.4103/ijpm.ijpm_259_24
TOLG, C.; COWMAN, M.; TURLEY, E. Mouse Mammary Gland Whole Mount Preparation and Analysis. BIO-PROTOCOL, v.8, n.13, 2018. doi: 10.21769/BioProtoc.2915
UNITED NATIONS. United Nations Department of Economy and Social Affairs. Population Division 2024. World Contraceptive Use 2024.
VALENTINE, J.M.; AHMADIAN, M.; KEINAN, O.; ABU-ODEH, M.; ZHAO, P.; ZHOU, X.; KELLER, M.P.; GAO, H.; YU, R.T.; LIDDLE, C.; DOWNES, M.; ZHANG, J.; LUSIS, A.J.; ATTIE, A.D.; EVANS, R.M.; RYDÉN, M.; SALTIEL, A.R. β3-Adrenergic receptor downregulation leads to adipocyte catecholamine resistance in obesity. Journal of Clinical Investigation, v.132, n.2, 2022. doi: 10.1172/JCI153357
WAWRZKIEWICZ-JAŁOWIECKA, A.; LALIK, A.; SOVERAL, G. Recent Update on the Molecular Mechanisms of Gonadal Steroids Action in Adipose Tissue. International Journal of Molecular Sciences, v.22, n.10, p.5226, 2021. doi: 10.3390/ijms22105226
WORLD OBESITY FEDERATION [Internet]. Prevalence of adult overweight & obesity. Acesso em: 24 ago. 2024. Disponível em: <https://data.worldobesity.org/tables/prevalence-of-adult-overweight-obesity-2/>.

RECEBIDO: 5/6/2025
ACEITO: 22/7/2025
PUBLICADO: 24/7/2025