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Impact of perinatal bisphenol A and 17β estradiol exposure: Comparing hormone receptor response

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ABSTRACT

Hormonal regulation controls mammary gland (MG) development. Therefore some hormone-related factors can disrupt the early phases of MGs development, making the gland more susceptible to long term modifications in its response to circulating hormones. Endocrine disruptors, such as bisphenol A (BPA), are able to cause alterations in hormone receptor expression, leading to changes in the cell proliferation index, which may expose the tissue to neoplastic alterations. Thus, we evaluated the variations in hormone receptor expression in the MG of 6-month old Mongolian gerbils exposed to BPA and 17β estradiol during the perinatal period. Receptors for estrogen alpha (ERα), beta (ERβ), progesterone (PGR), prolactin (PRL-R), and co-localization of connexin 43 (Cx43) and ERα in gerbils were analyzed, and serum concentrations of estradiol and progesterone were assessed. No alterations in body, liver, and ovary-uterus complex weights were observed. However, there was an increase in epithelial ERα expression in the 17β estradiol (E2) group and in PGR in the BPA group. Although immunohistochemistry did not show alterations in ERβ expression, western blotting revealed a decrease in this protein in the BPA group. PRL-R was more present in epithelial cells in the vehicle control (VC), E2, and BPA groups in comparison to the intact control group. Cx43 was more frequent in E2 and BPA groups, suggesting a protective response from the gland against possible malignancy. Serum concentration of estradiol reduced in VC, E2, and BPA groups, confirming that alterations also impacts steroid levels. Consequently, perinatal exposure to BPA and the reference endogenous estrogen, 17β estradiol, are able to increase the tendency of endocrine disruption in MG in a long term manner, since repercussions are observed even 6 months after exposure.

1. Introduction

The rise in human innovation and consequent industrialization necessitate technological adaptations. The development of practical materials, particularly plastic, was revolutionary and its benefits are undeniable in different areas, such as industry, medical compounds, and commodities (Kumar, 2018). However, the manufacturing of plastic must be rethought, according to the UN World Environment Day Outlook 2018, since huge amounts of plasticizers, elements added to polymeric materials that assist its processing and formulation (Pereira et al., 2015) are applied. The widely adopted compound bisphenol A (BPA) is frequently used for the manufacture of plastic and synthesis of epoxy resins (Andrady and Neal, 2009; Hoekstra and Simoneau, 2013).

BPA is known as an endocrine disruptor due to its capacity to mimic the effects of endogenous hormones (Taylor et al., 2011). It is also classified as a xenoestrogen, due to its capacity to bind to estrogen receptors (ERs) (Wetherill et al., 2007). Because of its structure, BPA is able to bind both ERα and ERβ. Since these are transcription factors, after ligand binding these receptors migrate to the nucleus, aiming to regulate the expression of estrogen target genes (Ascenzi et al., 2006). However, the post binding conformation of ERs may change according to the ligand, in such a way that xenoestrogens may promote

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"inappropriate" binding (Acconcia et al., 2015). These receptors play an important role in the growth and maintenance of a variety of tissues: mammary gland (MG), uterus, bone, and cardiovascular system (Delfosse et al., 2014). Both, ERα and ERβ are present in the MG and exert different functions. As a result, the proliferative and anti-proliferative effects of ERα and ERβ, respectively, may be disturbed due to impacts in the balance between their functions (Acconcia et al., 2015). Besides binding to ERs, BPA also exerts an impact in the expression of these receptors (Somogyi et al., 2016).

In addition, progesterone (PGR) (Krishnan et al., 1993; Li et al., 2016; Muñoz-de-Toro et al., 2005), androgen (Delfosse et al., 2012), growth hormone (Dang et al., 2009), and thyroid hormone receptors (Somogyi et al., 2016) may also be disrupted by BPA exposure. Some PGR dependent uterine functions have shown to be altered after prolonged BPA exposure (Li et al., 2016), affecting embryo implantation and decidualization during early pregnancy. In rats perinatally treated with BPA, increased induction of hormone function key mediators was observed (Durando et al., 2011). Prolactin release has also been demonstrated to be altered by BPA, since it mimics estradiol in inducing hyperprolactinemia after binding to ERs in the pituitary (Steinmetz et al., 1997).

It has been confirmed that even when xenoestrogen exposure occurs in the first stages of development, long term effects are present, with the adult individuals still being vulnerable to the consequences of perinatal exposure (Durando et al., 2006; Gomez et al., 2019; T.D et al., 2019; Muñoz-de-Toro et al., 2005). This effect is known as estrogenic imprint due to the long term consequences of estrogen exposure (Perez et al., 2016; Prins et al., 2007). Despite acting through different pathways, 17β estradiol has a more potent effect (Nomiri et al., 2019) since its estrogenic activity at a concentration of 10^{-8} M is similar to that of BPA at a concentration of 10^{-6} M (Hiroi et al., 1999). Thus, we used 17β estradiol as an endogenous estrogen control reference. Therefore, in this work we verified the influence of perinatal BPA and 17β estradiol exposure in the expression of hormonal receptors in the MGs of adult Mongolian gerbils, verifying alterations in hormone response later in life.

2. Material and methods

2.1. Ethics statement and environment

The procedures described for these experiments were conducted according to the standards defined by the National Council of Animal Experiment Control (CONCEA, Brazil, www.mctic.gov.br) and were approved by the Ethics Committee on the Use of Animals (CEUA) from IBILCE/UNESP (protocol number 113/2015). The animals were maintained in a BPA free isolator (Alesco, Monte Mor, SP, Brazil), placed in a rack with ventilation, in a controlled environment: room temperature of 23–26 °C, 50% relative humidity, and light/dark cycle of 12/12 h. Fresh water and a commercial chow (Presence, Paulínia, SP, Brazil) were provided *ad libitum* to all animals during the experiment. Before the experiment began, female Mongolian gerbils (*Meriones unguiculatus*) were housed with fertile males. Pregnancy was confirmed through the presence of sperm cells in the vaginal smears – pregnancy day 1. The offsprings obtained from treated pregnant/lactating animals were the focus of this experiment and were maintained under the same environmental conditions until euthanasia was performed.

2.2. Animals and experimental design

The pregnant gerbils were divided into four groups (n = 5). The first group was kept as the intact control group (C) and did not receive any treatment. The exposed groups were as follows: (VC) only corn oil (vehicle control; Mazola, Mairinque, SP, Brazil) daily; (E2) 17β estradiol (E8515, Sigma Aldrich, St. Louis, USA) 35 μg/kg (Pinto et al., 2008) diluted in 0.1 ml corn oil 3 times/week; and (BPA) 50 μg/kg BPA (239,658, Sigma Aldrich, St. Louis, USA) diluted in 0.1 ml corn oil daily (Vandenberg et al., 2012). The treatments were performed in the mothers from the 8th gestational day until the end of lactation (46 days of treatment); it was suspended only on the parturition day. The gavage was always performed during the morning (8–10 a.m.).

After weaning, one female from each offspring was randomly chosen and used for analysis (n = 5); other individuals obtained in the same offspring group were used in other experiments. The selected females were maintained under the previously described conditions until reaching 180 days of age. After this period, they were anesthetized with xylazine (3 mg/kg) and ketamine (10 mg/kg) and euthanized by decapitation. The euthanasia procedures were performed during the morning with the animals in the estrous phase of the cycle, indicated by vaginal smears.

2.3. Biometrical analysis

During the euthanasia procedure, the body weight was evaluated before decapitation. After dissection, the weights of the liver and ovary-uterus complex were obtained; the relative weight of these organs was calculated as a proportion, dividing the organ weight by the body weight. The organs were further evaluated for the possible presence of macroscopic lesions.

2.4. Histological processing and cytochemical staining

The MGs were collected for morphological and immunohistochemical (IHC) analysis. The right abdominal gland was carefully removed and immediately fixed in paraformaldehyde 4% for 24 h. Next, samples were transferred to ethanol 70% and histologically processed under a semi enclosed system (TP1020, Leica Biosystems, Buffalo Grove IL, USA). The paraffin blocks were sliced (4 μm thick) and the cuts placed on glass slides. Cytochemical staining was performed with hematoxylin and eosin (HE) for general morphological evaluation of the mammary tissue.

2.5. Immunohistochemistry

The primary antibodies used were anti-ERα (ERα, rabbit polyclonal, IgG, MC-20, sc-542, Santa Cruz Biotechnology, Dallas, TX, USA); anti-ERβ (ERβ, mouse polyclonal, IgG2a, B-1, sc-390243, Santa Cruz Biotechnology, Dallas, TX, USA); anti-PGR (Alpha PR6, mouse monoclonal, IgG2a, GTX22765; GeneTex, Irvine, CA, USA), and anti-PRL-R (Anti-Prolactin receptor U5, mouse monoclonal ab2772, Abcam, Cambridge, UK). Details on the solutions and protocols adopted are described in the Supplementary Table 1. In summary, antigen retrieval was performed, endogenous peroxidases were blocked by immersing the slides in 3% H_2O_2 at room temperature, and nonspecific proteins were blocked. The sections were incubated overnight at 4 °C with primary antibodies then secondary antibodies were applied for 1 h at room temperature. Positive staining was detected by using 3–30′-diaminobenzidine tetrahydrochloride (DAB) chromogen (NovolinkMaxDAB, RE7270-CE, Leica Biosystems, Buffalo Grove IL, USA) and counterstained with Mayer's hematoxylin.

Double IHC was also performed to check the concurrent expression of connexin 43 (Cx43) and ERα in cells of different MG compartments between the groups. For this protocol, slides were subjected to antigen retrieval in citrate buffer 0.1 M (75 min at 98 °C), endogenous peroxidase blockage in 0.3% H_2O_2 (30 min at RT), endogenous biotin blockage (Avidin/Biotin blocking system, SP-2001, Vector Laboratories, Burlingame, CA, USA), inhibition of unspecific proteins with protein concentrate stock solution (Mouse on Mouse basic KIT, BMK-2202, Vector Laboratories, Burlingame, CA, USA), and incubation with first primary antibody (1:100, mouse anti-connexin 43,CXN6, sc-59949, Santa Cruz Biotechnology, Dallas, TX, USA) at 4 °C overnight. A secondary anti-mouse biotinylated antibody (rabbit anti-mouse E0464,

Dako, Santa Clara, CA, USA) was used. The slides were then incubated with DAB for staining of Cx43. Immediately after, a second inhibition of unspecific proteins was performed, then the protocol was repeated: ERα antibody (1:250 rabbit monoclonal, NB300-560, Novus Biologicals, Centenial, CO, USA) was used for incubation overnight at 4 °C, secondary antibody coupled to alkaline phosphatase (1:100 goat anti-rabbit IgG H&L, ab97048, Abcam, Cambridge, UK) was applied for 60 min at RT and the revelation was performed with fast red (TR/Naphthol AS-MX Tablets, F4648, Sigma Aldrich, St. Louis, MO, USA).

2.6. Quantification of cell expression and intracellular localization of hormonal receptors

For ERα, ERβ, and PGR, 6 random fields per animal (n = 5, 30 fields/group) were digitized (400× magnification) using a B×61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120 Virtual Microscope Slide Scanning System (VS120–S5) from the same company. The relative frequency of epithelial cells with positive nuclei was calculated in the MG secretory epithelium. The percentage was calculated by dividing the number of positive nuclei by the total number of cells counted. The results were expressed as percentages.

2.7. Protein expression in mammary gland samples (western blotting)

The left abdominal MG was removed and frozen at −80 °C and the protein content extracted with a lysis buffer according to the protocol described by Li et al. (2010). In summary, samples were smashed and left for extraction (CelLytic MT cell lysis reagent, C3228, Sigma Aldrich St. Louis, MO, USA) at 4 °C during 1 h under agitation. The protease activity was blocked by adding an inhibitor (Protease Inhibitor Cocktail, P8340, Sigma Aldrich, St. Louis, MO, USA), then the samples were centrifuged for 20 min at 14,000 rpm, 4 °C. For quantification of protein concentration, a BCA Protein Assay Kit (Pierce BCA Protein Assay Kit, 23,227, Thermo Fischer Scientific, Rockford, IL, USA) was used and the absorbance was read in a microplate reader (SPECTROstar Omega, BMG Labtech, Ortenberg, Germany). The extracts were stored at −80 °C until analysis.

ERα, ERβ, and PGR were quantified in MG samples by western blotting. A total protein amount of 15 μg was placed in each well in the SDS page gel and subjected to electrophoresis (90 V for 120 min). The bands were transferred (100 V for 1 h) to a nitrocellulose membrane (Amersham Protram, 10,600,003, GE Healthcare, Darmstadt, Germany). Blots were blocked using skimmed milk 5% in TBS +0.1% Tween (TBSt) for 1 h at room temperature. Overnight incubation under agitation with anti-ERα, ERβ, PGR (previously described), and GAPDH (mouse monoclonal MB374 Millipore, 1:10,000) diluted in skimmed milk 1% in TBSt was performed. Subsequently, the membranes were washed in TBSt and incubated at room temperature for 1 h under agitation with horseradish peroxidase conjugated secondary antibodies (1:10,000 rabbit anti-mouse ab6728, Abcam, Cambridge, UK, or 1:10,000 goat anti-rabbit 115-035-144, Jackson Immunoresearch, Jannersville, PA, USA). After washing the membranes, antibody detection was revealed with ECL Substrate Pierce (32,109, ThermoFischer, Rockford, IL, USA) and Super Signal (34,094, ThermoFischer, Rockford, IL, USA) reagents. The membranes were revealed in an imaging system machine (ChemiDoc MP, BioRad, Hercules, CA, USA) and the band densities were analyzed in a densitometry program – Image J (version 1.52a, Wayne Rasband, NIH, USA).

2.8. Hormonal analysis

Blood was collected from the neck immediately after decapitation. Samples were centrifuged (1200 g, 20 min) and the serum was stored at −80 °C for later hormonal analysis. Estradiol and progesterone were quantified in duplicate by capture/sandwich ELISA using specific commercial kits, according to the manufacturer's instructions (estradiol EIA Kit, 582,251, Cayman Chemical Company, Ann Arbor, MI, USA and progesterone Accu-Bind ELISA Microwells, 4825–300, Monobind Inc., Lake Forest, CA, USA). The readings were performed in a SpectraMaxPlus 384 microplate reader (Molecular Devices, San Jose, CA, USA).

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). First, the data were checked for normality using the Kolmogorov-Smirnov test. Parametric data were analyzed by one-way ANOVA followed by Tukey's test. For non-parametric data, the Kruskal-Wallis test followed by Dunn's test was adopted. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Influence of disruptors on biometric parameters

The administration of compounds to mothers during gestation and lactation did not interfere in the weight of the body, ovary-uterus complex, or liver of daughters at 6 months of age (Table 1). Macroscopic lesions were not found either.

3.2. Influence of disruptors on hormone receptor expression and localization (immunohistochemistry)

The percentages of cells with positive nuclear staining are shown in Table 2. ERα increased in the E2 group (34.7 ± 3.4) in comparison to the C group (21.6 ± 2.8); the values found for VC and BPA groups did not demonstrate any differences in comparison with other groups (Fig. 1B). The percentages of ERβ positive cells, however, did not present any differences between the groups (Fig. 1D). The incidence of PGR (Fig. 1F) positive cells was higher in the BPA (21.4 ± 3.1) in comparison to the C group (8.2 ± 0.5).

The oscillating pattern of PRL-R expression did not allow for standard quantification of this protein. Thus, an evaluation of its expression pattern in both epithelial and stromal compartments between the groups was performed. The samples from C group showed PRL-R expression as an intense staining in cells from the adipose tissue (Fig. 2A). In this group, the secretory structures only showed positive staining in the fibroblasts (Fig. 2B), being rare in epithelial cells. In adipose tissue, only some fibroblasts (arrowheads) were positive (Fig. 2C), while adipocytes (arrows) did not stain for this protein (Fig. 2D). In the VC group, usually epithelial cells were positive for PRL-R; the epithelial staining, however presented a different pattern in comparison to fibroblasts, being diffuse and badly delimited (Fig. 2E). Both the E2 and BPA groups showed positive epithelial cells with the same pattern as seen in VC: the E2 group (Fig. 2F), however, apparently showed less

Table 1
Biometric data of Mongolian gerbils from Control (C), Vehicle Control (VC), Estradiol (E2) and Bisphenol A (BPA) groups. Data shown as mean ± SEM.

	C	VC	E2	BPA
Body weight (g)	71.6 ± 4.8	70.6 ± 1.5	68.5 ± 2.3	72.4 ± 2.4
Ovary-Uterus (g)	0.26 ± 0.04	0.27 ± 0.03	0.24 ± 0.04	0.28 ± 0.03
Ovary-Uterus relative ($x10^{-3}$)	3.63 ± 0.36	3.83 ± 0.52	3.51 ± 0.59	3.88 ± 0.44
Liver (g)	2.68 ± 0.17	2.74 ± 0.15	2.65 ± 0.11	3.01 ± 0.13
Liver relative ($x10^{-2}$)	3.78 ± 0.25	3.86 ± 0.15	3.88 ± 0.12	4.12 ± 0.11

Parametric data: liver and liver relative weight; non-parametric data: ovary-uterus and ovary-uterus relative weight.

Table 2
Percentages of positive cells for ERα, ERβ and PGR in the epithelium of Mongolian gerbils mammary gland from the Control (C), Vehicle Control (VC), Estradiol (E2) and Bisphenol A (BPA) groups. Data shown as mean ± SE. Asterisks indicate different results from the C group.

	C	VC	E2	BPA
ERα	21.6 ± 2.8	30.7 ± 2.1	34.7 ± 3.4*	31.2 ± 2.9
ERβ	50.9 ± 6.3	63.9 ± 6.3	57.3 ± 9.1	47.1 ± 6.7
PGR	8.2 ± 0.5	11.7 ± 2.2	15.5 ± 2.3	21.4 ± 3.1*

ANOVA was followed by Tukey's test for ERα and ERβ analysis (parametric data), and Kruskal-Wallis was followed by Dunn's test for PGR analysis (non-parametric data).

areas of positive epithelium in comparison to BPA (Fig. 2G). Although positive cells in adipose tissue were present in all groups, they were clearly less frequent in E2 and BPA samples.

3.3. Influence of disruptors on co-localization of ERα and connexin 43

Illustrative pictures of double IHC reaction for ERα and Cx43 are shown in Fig. 3. It was observed that Cx43 was constantly present in cells negative for ER-α. The expression of Cx43 was always cytoplasmic, present only in the periductal region; ER-α was predominantly nuclear, despite some rare staining being present in the cytoplasm of some cells (Fig. 3B'). Although no quantification was performed, higher availability of Cx43 cells was present in the treated groups, especially the BPA group.

3.4. Influence of disruptors on protein expression (western blotting)

The quantifications of estrogen and progesterone receptor protein expression are shown in Fig. 4. For ERα, a similar pattern as that observed in the IHC quantification was present: an increase from C to E2 and a lower concentration in BPA groups. Despite this similarity, the

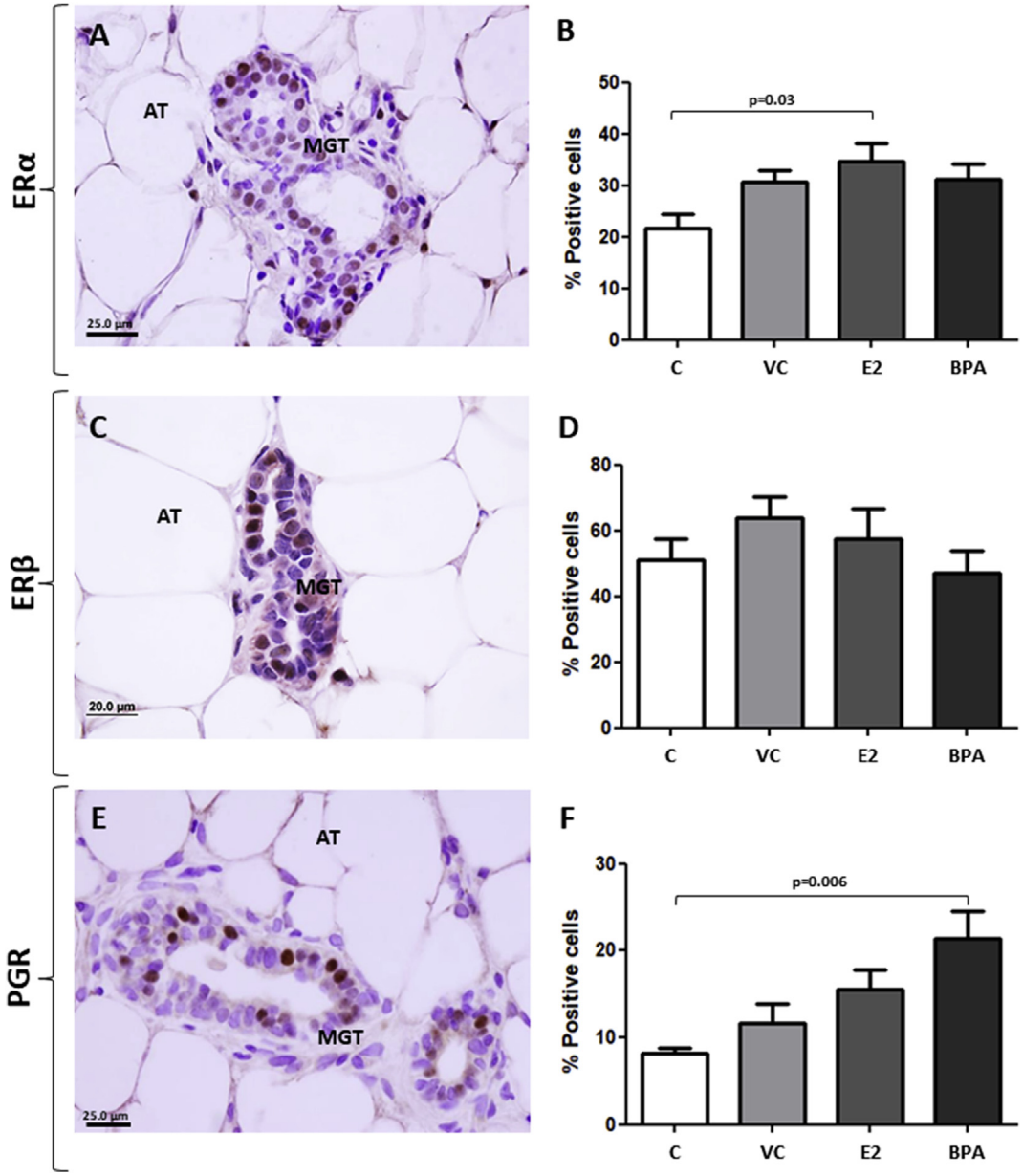

Fig. 1. Photomicrographs of nuclear receptors ERα (A, Estradiol group), ERβ (C, Vehicle Control group), and PGR (E, Bisphenol A group) immunostaining and their corresponding percentages of positive cells in the epithelium (B, D and F). Values expressed as mean ± SEM (ANOVA test followed by Tukey's test in B and D, Kruskal-Wallis test followed by Dunn's test in F). AT: adipose tissue; MGT: mammary gland tissue. Bars: 25 µm in A and E and 20 µm in C.

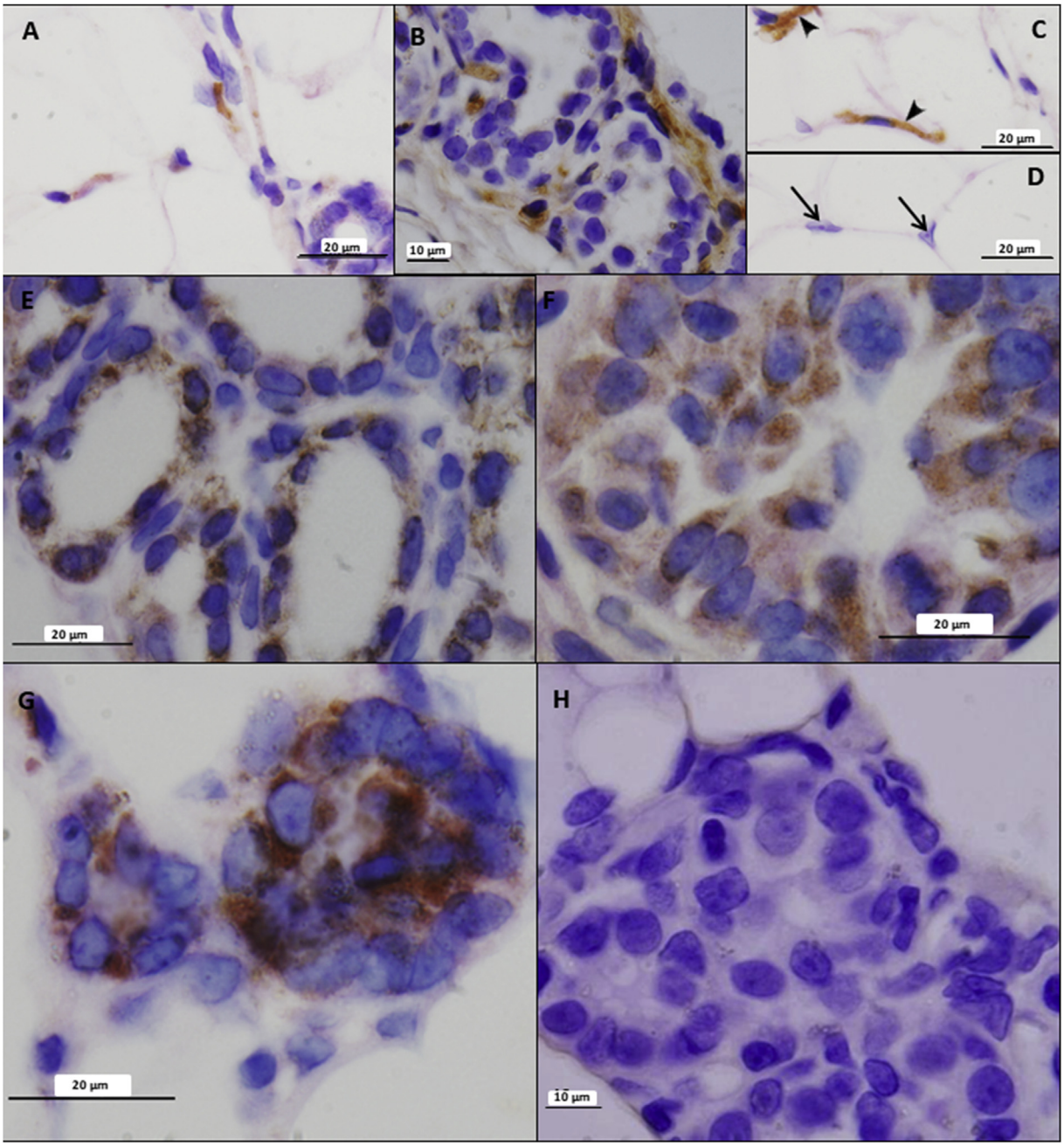

Fig. 2. PRL-R staining pattern in the Mongolian gerbil mammary gland from different groups. Control group showed a constant pattern of staining in the adipose tissue (A), surrounding the epithelial structures (B), specifically in fibroblasts (arrowheads) (C) but not in adipocytes (arrows) (D); in this group the staining of epithelial cells was rare. Vehicle Control (E), Estradiol (F) and Bisphenol A (G) groups showed epithelial structures usually presenting positive staining of epithelium. Negative control is shown in (H). Bars: 10 µm in B and H, and 20 µm in A, C-G.

relative concentration in BPA was considerably lower in comparison to VC and E2 groups. ERβ also demonstrated lower concentrations in the BPA, which differed statistically from E2. PGR expression, on the other hand, did not present differences between the groups.

3.5. Influence on serum estradiol and progesterone

The mean levels of serum estradiol are shown in Table 3. They were higher in the intact control group (328.8 ± 92.45 pg/ml) in comparison to the VC (126.7 ± 18.02 pg/ml), E2 (57.06 ± 11.16 pg/ml), and BPA (38.06 ± 12.89 pg/ml) groups. However, no differences were found between the C (4.37 ± 1.37 ng/ml), VC (7.41 ± 1.58 ng/ml), E2 (3.86 ± 1.01 ng/ml), and BPA (5.17 ± 1.24 ng/ml) groups for serum progesterone (Fig. 5).

4. Discussion

The experiments performed in the present work assessed the protein expression of ERα, ERβ, PGR and PRL-R. In terms of IHC, we found that the percentage of cells expressing ERα was higher in the 17β estradiol group in comparison to the control and that treatment with BPA increased the presence of PGR. Western blotting demonstrated differences between the BPA and 17β estradiol groups, not evidenced in the IHC analysis. Furthermore, different patterns of PRL-R and Cx43 expression were described among groups. Regarding the serum concentrations of estradiol, there were significant decreases in all groups in comparison

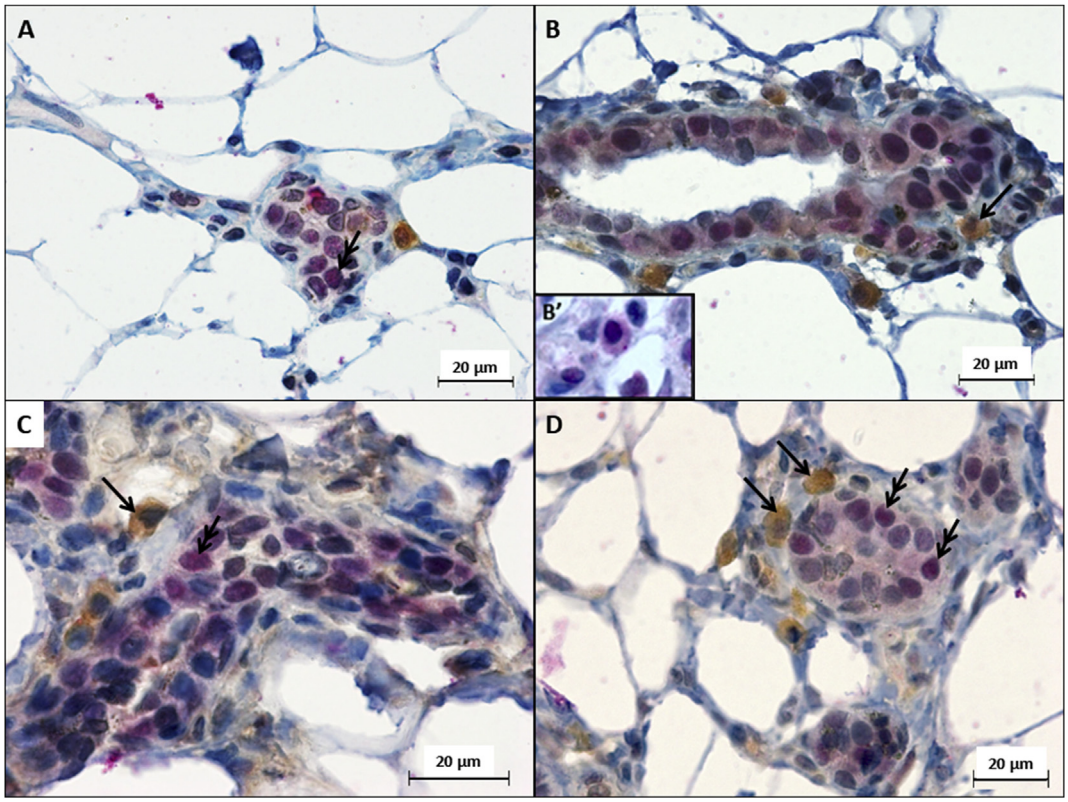

Fig. 3. Photomicrographs from Mongolian gerbil mammary gland subjected to double immunohistochemistry with Cx43 (arrows – brown staining) and ERα (double arrows – pink staining) in Control (A), Vehicle Control (B), Estradiol (C), and Bisphenol A (D) groups. There was no protein expression overlapping in the cells. Some cytoplasmic staining of ERα was observed (B′). Bars: 20 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to the intact control. In summary, the findings described above showed that perinatal exposure to xenoestrogen BPA and 17β estradiol is able to cause molecular alterations in the MG and fluctuations in the serum levels of estrogen, suggesting considerable long-term repercussions, as observed in other experiments (Acevedo et al., 2018; Mandrup et al., 2013).

Despite the known obesogenic effect of some endocrine disruptors (Grün and Blumberg, 2006), body weight presented no significant changes between the groups; in the same way, the ovary-uterus and liver weights did not change. Contrarily, at 21 weeks of age, rats showed higher body weight after perinatal BPA treatment (Xia et al., 2014). Despite this, the authors did not observe any differences in absolute or relative liver weights between treated and control animals, as seen in our results. Increases in liver weight due to hepatotoxic injuries may be caused by inflammation related to adduct formation, leading to hepatic scar depositions in long term or proliferative lesions (Zachary and McGavin, 2013). In addition, the challenge of BPA metabolism during fetal life may cause adult metabolic diseases as observed in other experiments with rodents (Le Magueresse-Battistoni et al., 2018). Our evaluations suggest that the treatment adopted was not sufficient to cause these systemic alterations in Mongolian gerbils, not even in terms of the female reproductive tract, as demonstrated by similar weights of ovary-uterus complexes.

Contrarily, steroid hormone receptors showed responses to the treatments. Their expression in mammary tumors is usually a biological marker for prognosis prediction and treatment decisions. More specifically, ERα and PGR have been linked to breast cancer risk in humans (Burns and Korach, 2012; Khan et al., 1998). However, the relation of both receptor expressions was not observed in a study performed a decade ago, where the authors found that, among postmenopausal women, breast cancer risk was inversely associated with expression of ERα and PGR (Lagiou et al., 2009).

In Mongolian gerbils, to date, western blotting analysis of hormone receptors is limited to some organs, such as the testes (Negrin et al., 2018) uterus, and stomach (Saqui-Salces et al., 2008); thus, this is the first description of ERα, ERβ, and PGR protein bands detection in MG in this rodent species. Bands of 68 kDa were detected for ERα, 56 kDa for ERβ, and 120 kDa for PGR, suggesting the exclusive detection of PGR-B isoform (Datasheet: Progesterone Receptor antibody [Alpha PR6], Genetex, 2019). With the exception of ERβ, the other receptors have been described in gerbils by Saqui-Salces et al. (2008), who could not detect ERβ bands and found 85 kDa PGR bands, indicating PGR-A isoform. These differences may have been due to the use of different antibodies and evaluation of different tissues; or, alternatively, this may represent a specificity of Mongolian gerbils.

In the present experiment IHC analysis showed lower expression of ERα in the intact control in comparison to the 17β estradiol treated group; however, no difference was present when compared to the BPA exposed group. Western blotting, in contrast, was able to evidence that the slight reduction in ERα expression in the BPA shown by IHC is significant when the whole tissue is evaluated. Since our IHC quantification was performed exclusively in the epithelial compartment, it is possible that the ERα expression in other mammary components was significantly higher in VC and E2 groups. Indeed, positive cells for ERs and PGR were present in the stromal compartment. For PGR, no different results were found, showing that this receptor did not vary between the groups as an effect of BPA or 17β estradiol. ERβ, however, showed lower expression in BPA treated animals. In terms of human disease, no correlation was demonstrated for ERβ and breast cancer malignity (Borgquist et al., 2008). Furthermore, the expression pattern of this receptor tends to be higher in the involution phases, as previously described for Mongolian gerbils (Leonel et al., 2017), suggesting

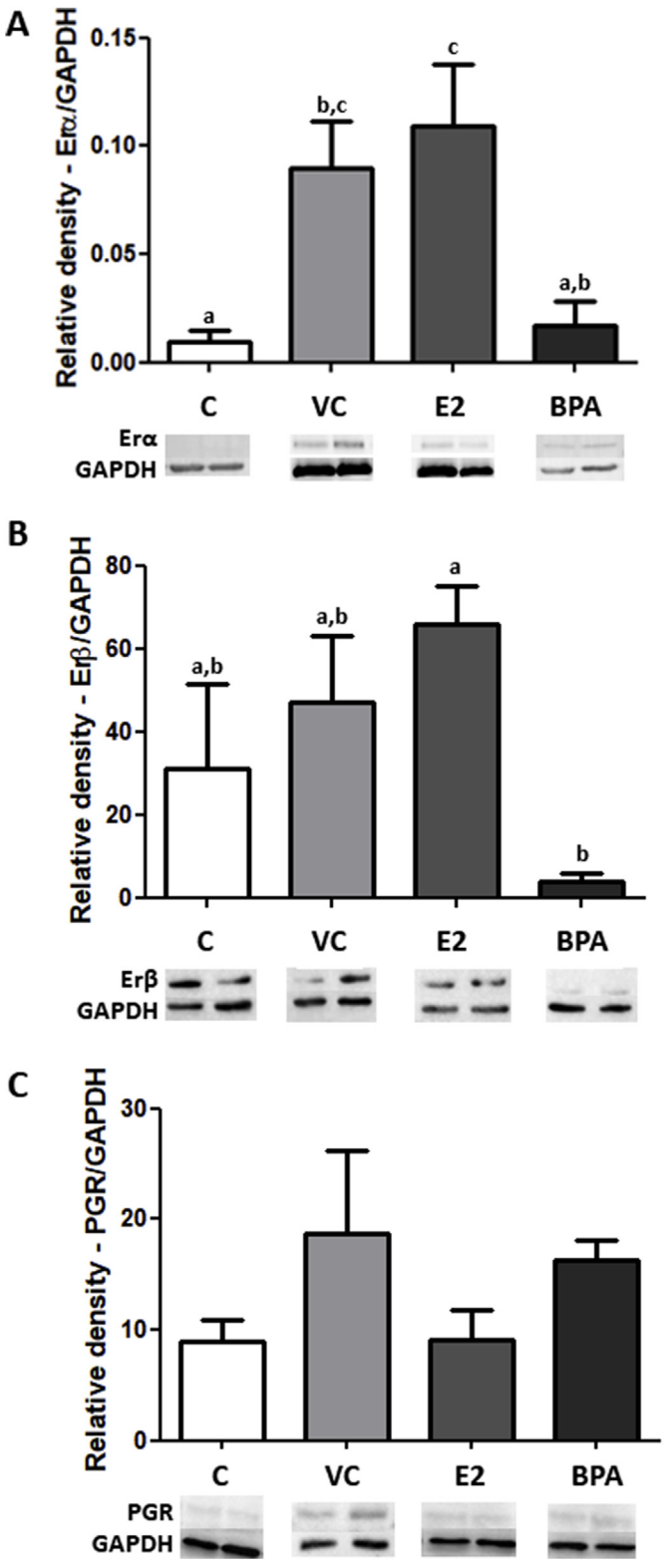

Fig. 4. Relative density of ERα (A), ERβ (B), and PGR (C) normalized to GAPDH, here used as positive control. Same letters above bars indicate similar results (n = 4 animals per group). Values are expressed as mean ± SEM, $p < 0.05$ (ANOVA followed by Tukey's test).

that this receptor is not associated with MG proliferation. In fact, it has previously been stated that ERβ serves to facilitate ERα return to the nucleus, restoring its responsiveness to estrogens (Cheng et al., 2004), and does not relate to proliferative malignancy of the MG epithelium.

Table 3
Levels of serum estradiol and progesterone of Mongolian gerbils from the Control (C), Vehicle Control (VC), Estradiol (E2) and Bisphenol A (BPA) groups. Data shown as mean ± SE. Asterisk indicate different result from the C group.

	C	VC	E2	BPA
Estradiol (pg/ml)	328.8 ± 92,4	126.7 ± 18.0	57.0 ± 11.1*	38.0 ± 12.8
Progesterone (ng/ml)	4.3 ± 1,3	7.4 ± 1.5	3.8 ± 1.0	5.1 ± 1.2

ANOVA followed by Tukey's test.

Regarding the pattern of expression, in humans, ERα and PGR tend to be more localized in epithelial cells, whereas ERβ tends to be more widespread (Burns and Korach, 2012; Li et al., 2010). Our analysis by IHC and blots showed similar fluctuation patterns for both ERα and ERβ between the groups. However, the statistical difference observed in ERβ blots between BPA and E2 suggests that this receptor is more expressed in the non-epithelial compartment of the gland in E2. According to Paech et al. (1997), the presence of estrogen makes ERα an activator and ERβ an inhibitor of transcription. However, it was suggested that ERβ plays the role of an independent regulator, with distinct cellular functions (Saji et al., 2000), including a protective role since this receptor can down-regulate ERα transcription capacity (Paech et al., 1997). In this line, since ERβ is normally more abundantly expressed in rodent MG (Saji et al., 2000), its reduction in the BPA may indicate a malignant effect caused by perinatal exposure to BPA.

Contrary to estrogen and progesterone receptors, PRL-R showed different patterns of expression, including nuclear and cytoplasmic reactivity. Thus, we described its cell localization and compared it with data previously described in the literature for other species, since no information regarding Mongolian gerbils was available. The presence of PRL-R in epithelial cells increases during pregnancy and lactation in the rat model, suggesting its necessity during proliferation and milk production MG phases (Camarillo et al., 2001). In summary, that work stated that stromal expression of PRL-R did not change among different developmental stages; epithelial expression, however, considerably increases in pregnancy and lactation, leading one to conclude that prolactin action shifts to epithelium in fully differentiated MG cells. The constant presence of PRL-R in the fat pad may be explained by its regulation of lipoprotein lipase (Hang and Rillema, 1997), an important enzyme for fatty acid metabolism. Further experiments, however, must be performed on this point of interest to answer this question.

The Cx43 MG malignancy marker, also known as gap junction protein alpha 1, although normally localized in cell junctions is also found in vesicles in the cytoplasm and nucleoplasm (Thul et al., 2017). Even though this protein function is normally a component of gap junctions, we could not find it in the epithelial cells, as expected. The idea that Cx43 is linked to MG malignancy (Plante et al., 2011) led us to evaluate the relation between its expression and that of ERα, a marker for cells that respond to estradiol and tend to proliferate. However, Cx43 was only present in cells not expressing ERα, normally present in epithelial cells. In fact, the family of gap junction proteins, in addition to intercellular communication, is involved with cell proliferation, cell differentiation, and the cell homeostasis phenomenon (Hervé et al., 2007). Previous experiments described the expression of Cx43 in myoepithelial cells (Pozzi et al., 1995; Yamanaka et al., 1997), suggesting that they were interconnected by gap junctions formed by Cx43. According to Yamanaka et al. (1997), this expression increases right after delivery in myoepithelial cells, suggesting an important role of Cx43 in the lactating process (Plum et al., 2000). The expression of Cx43 in the Mongolian gerbil MG was present as punctate plaques, as previously described (Stewart et al., 2013). The presence of Cx43 in the cytoplasm suggests preparation for cells that will need more contraction abilities, such as its pattern of expression in the endometrium during pregnancy, which increases in preparation for birth but is concentrated

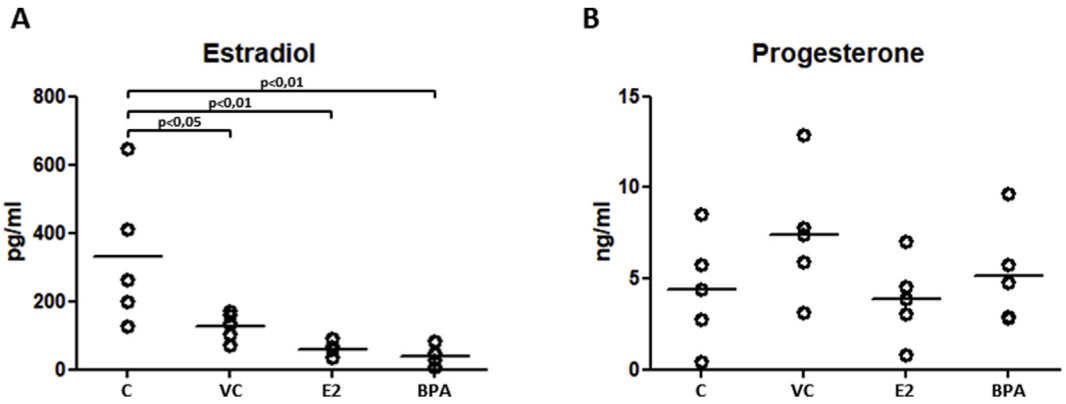

Fig. 5. Serum hormonal profiles in Mongolian gerbils from different experimental groups. The estradiol (A) and progesterone (B) dosages were performed in 5 animals per group. Individual values (circles) and mean values (lines) are shown. Values were considered significant when $p < 0.05$ (ANOVA test followed by Tukey's test).

in the cytoplasm; at the time of birth, it migrates to the plasma membrane, where it will exert its function (Hendrix et al., 1992). Thus, an apparent increase in Cx43 positive cells in the BPA may be related to a protective response of the gland due to a possible risk environment, since Cx43 is frequently present in stroma of invasive carcinomas (Jamieson et al., 1998; McLachlan et al., 2007).

Despite some experiments reporting the increase in serum estradiol after BPA treatment in mice (Xi et al., 2011) and in rats (Fernández et al., 2010) we found a considerable decrease in this parameter. Xi et al. (2011) assume that the effects of perinatal BPA administration cause alterations in gonad steroidogenesis; however, these authors evaluated pups, while Fernández et al. (2010) analyzed the serum from 4 to 5 month old rats. These authors assumed that the high estrogen levels found were due to the presence of ovarian cysts. These cysts may increase the serum levels of BPA (Crain et al., 2008) since they may cause a reduction in BPA clearance (Diamanti-Kandarakis et al., 2009). The decrease in serum estradiol levels after perinatal treatment with BPA and ethinyl estradiol has been observed in rats (Patel et al., 2017), similarly to that described in the present experiment. A presumable explanation for the increase in estradiol levels in that experiment is the route of exposure, subcutaneous, which may result in the absorption of higher levels of BPA in comparison to the oral route. It is important to state that in our experiment, euthanasia was always performed in the same phase of the estrous cycle, avoiding changes due to the natural cyclic variation in hormonal production.

The alterations observed in the VC group were not expected. Despite this oil has been applied as a vehicle for lipophilic compounds (Bostanci et al., 2016; Garcia et al., 2015) its long-term effect was closer to the treated groups in terms of hormone receptor expression and serum levels of estradiol. As previously stated, administration of lipid compounds may cause a stress response (Brown et al., 2000) as well as alterations in gene expression profiles (Geng et al., 2012). Still, estrogenic isoflavones are known to be present in genetically modified corn (Schettler, 2003). It was proposed that n-6 fatty acids present in corn oil may have developmental impacts (Hilakivi-Clarke et al., 1997). However the exact identity of substances with hormonal activity present in corn is still unknown. We believe that corn oil applied alone changed patterns of protein expression. Experiments aiming to unveil lipophilic and hydrophilic vehicles mechanism in different tissues are being developed in our laboratory.

In order to answer the main question of this work, one should speculate on the results to evaluate similarities and differences between the mechanisms of action of BPA and 17β estradiol. The endogenous estradiol mechanism of action is well known and is based exclusively on receptor binding. BPA, however may have an action after binding to different receptors, such as peroxisome proliferator-activated receptor γ (Delfosse et al., 2014), to non-canonical estrogen receptors not localized in the nucleus (Marino et al., 2012), or even inhibit the action of androgen receptors (Delfosse et al., 2014). Thus, even though we could not specify the actual mechanism of action, the effects of perinatal exposure to BPA and 17β estradiol in the adult female MG are distinct and peculiar for this species. Furthermore, the decrease in serum estrogen may have led to modulation or disruption of the estrogenic pathway in adult female gerbils exposed during neonatal life.

In conclusion, our results indicate that perinatal exposure to BPA and 17β estradiol may alter the expression of ERs, allowing hormonal disruption in adulthood; the decrease in serum estradiol levels supports this conclusion. Exposure also affects the levels of PGR epithelial expression and the localization pattern of PRL-R, also in addition to the responses to progesterone and prolactin in the MG of female gerbils.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109918.

References

Acconcia, F., Pallottini, V., Marino, M., 2015. Molecular mechanisms of action of BPA. Dose-Response 13https://doi.org/10.1177/1559325815610582. 1559325815610582.

Acevedo, N., Rubin, B.S., Schaeberle, C.M., Soto, A.M., 2018. Perinatal BPA exposure and reproductive axis function in CD-1 mice. Reprod. Toxicol. 79, 39–46. https://doi.org/10.1016/j.reprotox.2018.05.002.

Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. Biol. Sci. 364 (1526), 84–1977.

Ascenzi, P., Bocedi, A., Marino, M., 2006. Structure–function relationship of estrogen

receptor α and β: impact on human health. Mol. Asp. Med. 27, 299–402.
Borgquist, S., Holm, C., Stendahl, M., Anagnostaki, L., Landberg, G., Jirström, K., 2008. Oestrogen receptors α and β show different associations to clinicopathological parameters and their co-expression might predict a better response to endocrine treatment in breast cancer. J. Clin. Pathol. 61https://doi.org/10.1136/jcp.2006.040378. 197 LP – 203.
Bostanci, Z., Mack, R.P., Enomoto, L.M., Alam, S., Brown, A., Neumann, C., Soybel, D.I., Kelleher, S.L., 2016. Marginal zinc intake reduces the protective effect of lactation on mammary gland carcinogenesis in a DMBA-induced tumor model in mice. Oncol. Rep. 35, 1409–1416.
Brown, A.P., Dinger, N., Levine, B.S., 2000. Stress produced by gavage administration in the rat. J. Am. Assoc. Lab. Anim. Sci. 39, 17–21.
Burns, K.A., Korach, K.S., 2012. Estrogen receptors and human disease: an update. Arch. Toxicol. 86, 1491–1504.
Camarillo, I.G., Thordarson, G., Moffat, J.G., Van Horn, K.M., Binart, N., Kelly, P.A., Talamantes, F., 2001. Prolactin receptor expression in the epithelia and stroma of the rat mammary gland. J. Endocrinol. 171, 85–95.
Cheng, G., Weihua, Z., Warner, M., Gustafsson, J.-Å., 2004. Estrogen receptors ERα and ERβ in proliferation in the rodent mammary gland. Proc. Natl. Acad. Sci. U.S.A. 101, 3739. https://doi.org/10.1073/pnas.0307864100. LP – 3746.
Crain, D.A., Janssen, S.J., Edwards, T.M., Heindel, J., Ho, S., Hunt, P., Iguchi, T., Juul, A., McLachlan, J.A., Schwartz, J., 2008. Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. Fertil. Steril. 90, 911–940.
Dang, V.H., Choi, K.-C., Jeung, E.-B., 2009. Estrogen receptors are involved in xenoestrogen induction of growth hormone in the rat pituitary gland. J. Reprod. Dev. 55, 206–213.
Datasheet Progesterone Receptor antibody [Alpha PR6], Genetex, 2019. https://www.genetex.com/PDF/Download?catno=GTX22765, Accessed date: 22 August 2019.
Delfosse, V., Grimaldi, M., Pons, J.-L., Boulahtouf, A., le Maire, A., Cavailles, V., Labesse, G., Bourguet, W., Balaguer, P., 2012. Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. Proc. Natl. Acad. Sci. 109https://doi.org/10.1073/pnas.1203574109. 14930 LP – 14935.
Delfosse, V., Grimaldi, M., Le Maire, A., Bourguet, W., Balaguer, P., 2014. Nuclear receptor profiling of bisphenol-A and its halogenated analogues. In: Vitamins & Hormones. Elsevier, pp. 229–251.
Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. 30, 293–342.
Durando, M., Kass, L., Piva, J., Sonnenschein, C., Soto, A.M., Luque, E.H., Muñoz-De-Toro, M., 2006. Prenatal bisphenol a exposure induces preneoplastic lesions in the mammary gland in wistar rats. Environ. Health Perspect. 115, 80–86. https://doi.org/10.1289/ehp.9282.
Durando, M., Kass, L., Perdomo, V., Bosquiazzo, V.L., Luque, E.H., Muñoz-De-Toro, M., 2011. Prenatal exposure to bisphenol A promotes angiogenesis and alters steroid-mediated responses in the mammary glands of cycling rats. J. Steroid Biochem. Mol. Biol. 127, 35–43. https://doi.org/10.1016/j.jsbmb.2011.04.001.
Fernández, M., Bourguignon, N., Lux-Lantos, V., Libertun, C., 2010. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environ. Health Perspect. 118, 1217.
Garcia, C.P., Lamarque, A.L., Comba, A., Berra, M.A., Silva, R.A., Labuckas, D.O., Das, U.N., Eynard, A.R., Pasqualini, M.E., 2015. Synergistic anti-tumor effects of melatonin and PUFAs from walnuts in a murine mammary adenocarcinoma model. Nutrition 31, 570–577. https://doi.org/10.1016/j.nut.2014.06.001.
Geng, X., Li, B., Zhang, L., Song, Y., Lin, Z., Zhang, Y., Wang, J., 2012. Corn oil as a vehicle in drug development exerts a dose-dependent effect on gene expression profiles in rat thymus. J. Appl. Toxicol. 32, 850–857.
Gomez, A.L., Altamirano, G.A., Leturia, J., Bosquiazzo, V.L., Muñoz-de-Toro, M., Kass, L., 2019. Male mammary gland development and methylation status of estrogen receptor alpha in Wistar rats are modified by the developmental exposure to a glyphosate-based herbicide. Mol. Cell. Endocrinol. 481, 14–25. https://doi.org/10.1016/j.mce.2018.11.005.
Grün, F., Blumberg, B., 2006. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 147, s50–s55.
Hang, J., Rillema, J.A., 1997. Prolactin's effects on lipoprotein lipase (LPL) activity and on LPL mRNA levels in cultured mouse mammary gland explants. Proc. Soc. Exp. Biol. Med. 214, 161–166.
Hendrix, E.M., Mao, S.J.T., Everson, W., Larsen, W.J., 1992. Myometrial connexin 43 trafficking and gap junction assembly at term and in preterm labor. Mol. Reprod. Dev. 33, 27–38.
Hervé, J.-C., Bourmeyster, N., Sarrouilhe, D., Duffy, H.S., 2007. Gap junctional complexes: from partners to functions. Prog. Biophys. Mol. Biol. 94, 29–65.
Hilakivi-Clarke, L., Clarke, R., Onojafe, I., Raygada, M., Cho, E., Lippman, M., 1997. A maternal diet high in n-6 polyunsaturated fats alters mammary gland development, puberty onset, and breast cancer risk among female rat offspring. Proc. Natl. Acad. Sci. U.S.A. 94 (17), 9372–9377.
Hiroi, H., Tsutsumi, O., Momoeda, M., Takai, Y., Osuga, Y., Taketani, Y., 1999. Differential interactions of bisphenol A and17β-estradiol with estrogen receptor α (ERα) and ERβ. Endocr. J. 46, 773–778.
Hoekstra, E.J., Simoneau, C., 2013. Release of bisphenol A from polycarbonate—a review. Crit. Rev. Food Sci. Nutr. 53, 386–402.
Jamieson, S., Going, J.J., D'Arcy, R., George, W.D., 1998. Expression of gap junction proteins connexin 26 and connexin 43 in normal human breast and in breast tumours. J. Pathol. A J. Pathol. Soc. Gt. Britain Irel. 184, 37–43.
Khan, S.A., Rogers, M.A.M., Khurana, K.K., Meguid, M.M., Numann, P.J., 1998. Estrogen receptor expression in benign breast epithelium and breast cancer risk. JNCI J. Natl. Cancer Inst. 90, 37–42.
Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L., Feldman, D., 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132, 2279–2286.
Kumar, P., 2018. Role of plastics on human health. Indian J. Pediatr. 85, 384–389. https://doi.org/10.1007/s12098-017-2595-7.
Lagiou, P., Georgila, C., Samoli, E., Lagiou, A., Zourna, P., Minaki, P., Vassilarou, D., Papadiamandis, I., Sfikas, C., Kalapothaki, V., Sekeris, C.E., Trichopoulos, D., 2009. Estrogen alpha and progesterone receptor expression in the normal mammary epithelium in relation to breast cancer risk. Int. J. Cancer 124, 440–442. https://doi.org/10.1002/ijc.23899.
Le Magueresse-Battistoni, B., Multigner, L., Beausoleil, C., Rousselle, C., 2018. Effects of bisphenol A on metabolism and evidences of a mode of action mediated through endocrine disruption. Mol. Cell. Endocrinol.
Leonel, E.C.R., Falleiros, L.R., Campos, S.G.P., Taboga, S.R., 2017. Histological and immunohistochemical characterization of the Mongolian gerbil's mammary gland during gestation, lactation and involution. Acta Histochem. 119 (3), 273–283. https://doi.org/10.1016/j.acthis.2017.02.003.
Li, N., Dong, J., Hu, Z., Shen, H., Dai, M., 2010. Potentially functional polymorphisms in ESR1 and breast cancer risk: a meta-analysis. Breast Canc. Res. Treat. 121, 177–184. https://doi.org/10.1007/s10549-009-0532-9.
Li, Q., Davila, J., Bagchi, M.K., Bagchi, I.C., 2016. Chronic exposure to bisphenol a impairs progesterone receptor-mediated signaling in the uterus during early pregnancy. Recept. Clin. Investig. 3.
Mandrup, K.R., Jacobsen, P.R., Isling, L.K., Axelstad, M., Dreisig, K., Hadrup, N., Vinggaard, A.M., Hass, U., Boberg, J., 2013. Effects of perinatal ethinyl estradiol exposure in male and female Wistar rats. Reprod. Toxicol. 42, 180–191. https://doi.org/10.1016/j.reprotox.2013.09.001.
Marino, M., Pellegrini, M., La Rosa, P., Acconcia, F., 2012. Susceptibility of estrogen receptor rapid responses to xenoestrogens: physiological outcomes. Steroids 77, 910–917.
McLachlan, E., Shao, Q., Laird, D.W., 2007. Connexins and gap junctions in mammary gland development and breast cancer progression. J. Membr. Biol. 218, 107–121.
Muñoz-de-Toro, M., Markey, C.M., Wadia, P.R., Luque, E.H., Rubin, B.S., Sonnenschein, C., Soto, A.M., 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146, 4138–4147. https://doi.org/10.1210/en.2005-0340.
Negrin, A.C., de Jesus, M.M., Christante, C.M., da Silva, D.G.H., Taboga, S.R., Pinto-Fochi, M.E., Góes, R.M., 2018. Maternal supplementation with corn oil associated or not with di-n-butyl phthalate increases circulating estradiol levels of gerbil offspring and impairs sperm reserve. Reprod. Toxicol. 81, 168–179.
Nomiri, S., Hoshyar, R., Ambrosino, C., Tyler, C.R., Mansouri, B., 2019. A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. Environ. Sci. Pollut. Res. 26, 8459–8467.
Paech, K., Webb, P., Kuiper, G.G.J.M., Nilsson, S., Gustafsson, J.-Å., Kushner, P.J., Scanlan, T.S., 1997. Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science 277 (80), 1508–1510.
Patel, S., Brehm, E., Gao, L., Rattan, S., Ziv-Gal, A., Flaws, J.A., 2017. Bisphenol a exposure, ovarian follicle numbers, and female sex steroid hormone levels: results from a CLARITY-BPA study. Endocrinology 158, 1727–1738.
Pereira, L.C., de Souza, A.O., Bernardes, M.F.F., Pazin, M., Tasso, M.J., Pereira, P.H., Dorta, D.J., 2015. A perspective on the potential risks of emerging contaminants to human and environmental health. Environ. Sci. Pollut. Res. 22, 13800–13823. https://doi.org/10.1007/s11356-015-4896-6.
Perez, A.P.S., Biancardi, M.F., Caires, C.R.S., Falleiros-Junior, L.R., Góes, R.M., Vilamaior, P.S.L., Santos, F.C.A., Taboga, S.R., 2016. Prenatal exposure to ethinylestradiol alters the morphologic patterns and increases the predisposition for prostatic lesions in male and female gerbils during ageing. Int. J. Exp. Pathol. 97, 5–17. https://doi.org/10.1111/iep.12153.
Pinto, M.E., Vilamaior, P.S.L., Taboga, S.R., Góes, R.M., 2008. Exposure of young rats to high estrogen doses leads to degeneration of elongated spermatids. Tissue Cell 40, 31–42. https://doi.org/10.1016/j.tice.2007.09.002.
Plante, I., Stewart, M.K.G., Barr, K., Allan, A.L., Laird, D.W., 2011. Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease. Oncogene 30, 1681.
Plum, A., Hallas, G., Magin, T., Dombrowski, F., Hagendorff, A., Schumacher, B., Wolpert, C., Kim, J.S., Lamers, W.H., Evert, M., 2000. Unique and shared functions of different connexins in mice. Curr. Biol. 10, 1083–1091.
Pozzi, A., Risek, B., Kiang, D.T., Gilula, N.B., Kumar, N.M., 1995. Analysis of multiple gap junction gene products in the rodent and human mammary gland. Exp. Cell Res. 220, 212–219. https://doi.org/10.1006/excr.1995.1308.
Prins, G.S., Birch, L., Tang, W.-Y., Ho, S.-M., 2007. Developmental estrogen exposures predispose to prostate carcinogenesis with aging. Reprod. Toxicol. 23, 374–382. https://doi.org/10.1016/j.reprotox.2006.10.001.
Saji, S., Jensen, E.V., Nilsson, S., Rylander, T., Warner, M., Gustafsson, J. a, 2000. Estrogen receptors alpha and beta in the rodent mammary gland. Proc. Natl. Acad. Sci. U.S.A. 97, 337–342.
Saqui-Salces, M., Neri-Gómez, T., Gamboa-Dominguez, A., Ruiz-Palacios, G., Camacho-Arroyo, I., 2008. Estrogen and progesterone receptor isoforms expression in the stomach of Mongolian gerbils. World J. Gastroenterol. WJG 14, 5701.
Schettler, T., 2003. Corn and corn-derived products: sources of endocrine disruptors. Environ. Health Perspect. 111 (13), A691.
Somogyi, V., Horváth, T.L., Tóth, I., Bartha, T., Frenyó, L.V., Kiss, D.S., Jócsák, G., Kerti, A., Naftolin, F., Zsarnovszky, A., 2016. Bisphenol A influences oestrogen-and thyroid hormone-regulated thyroid hormone receptor expression in rat cerebellar cell

culture. Acta Vet. Hung. 64, 497–513.
Steinmetz, R., Brown, N.G., Allen, D.L., Bigsby, R.M., Ben-Jonathan, N., 1997. The environmental estrogen bisphenol a stimulates prolactin release in vitro and in vivo*. Endocrinology 138, 1780–1786.
Stewart, M.K.G., Gong, X.-Q., Barr, K.J., Bai, D., Fishman, G.I., Laird, D.W., 2013. The severity of mammary gland developmental defects is linked to the overall functional status of Cx43 as revealed by genetically modified mice. Biochem. J. 449https://doi.org/10.1042/BJ20121070. 401 LP – 413.
Taylor, J.A., Richter, C.A., Ruhlen, R.L., vom Saal, F.S., 2011. Estrogenic environmental chemicals and drugs: mechanisms for effects on the developing male urogenital system. J. Steroid Biochem. Mol. Biol. 127, 83–95. https://doi.org/10.1016/j.jsbmb.2011.07.005.
TD, K., Schantel, H.B., BS, S., KG, E., FS, E., 2019. Evaluation of prenatal exposure to bisphenol analogues on development and long-term health of the mammary gland in female mice. Environ. Health Perspect. 126, 87003. https://doi.org/10.1289/EHP3189.
Thul, P.J., Åkesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Blal, H.A., Alm, T., Asplund, A., Björk, L., Breckels, L.M., 2017. A subcellular map of the human proteome. Science 356 (80), eaal3321.
Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.
Wetherill, Y.B., Akingbemi, B.T., Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., Watson, C.S., Zoeller, R.T., Belcher, S.M., 2007. In vitro molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24, 178–198. https://doi.org/10.1016/j.reprotox.2007.05.010.
Xi, W., Lee, C.K.F., Yeung, W.S.B., Giesy, J.P., Wong, M.H., Zhang, X., Hecker, M., Wong, C.K.C., 2011. Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus–pituitary–gonadal axis of CD-1 mice. Reprod. Toxicol. 31, 409–417.
Xia, W., Jiang, Y., Li, Y., Wan, Y., Liu, J., Ma, Y., Mao, Z., Chang, H., Li, G., Xu, B., 2014. Early-life exposure to bisphenol a induces liver injury in rats involvement of mitochondria-mediated apoptosis. PLoS One 9, e90443.
Yamanaka, I., Kuraoka, A., Inai, T., Ishibashi, T., Shibata, Y., 1997. Changes in the phosphorylation states of connexin43 in myoepithelial cells of lactating rat mammary glands. Eur. J. Cell Biol. 72, 166–173.
Zachary, J.F., McGavin, M.D., 2013. Pathologic Basis of Veterinary Disease. E-Book.