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Dehydroepiandrosterone sulphate (DHEAS) supplementation impacts adrenal cortex morphophysiology of aged female gerbils

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ABSTRACT

During the process of aging, it is common for women to take dehydroepiandrosterone sulphate (DHEAS) supplements to prevent adrenopause. However, the potential effects of this supplementation on the adrenal cortex have not yet been fully elucidated. Therefore, the present study aimed to analyze the effects of DHEAS supplementation on the adrenal cortex of female Mongolian gerbils during the aging process. The experiment was conducted by dividing the aged female gerbils (18 months of age) into two groups (n = 5). The control group received no treatment, while the experimental group received 60 mg/kg of DHEAS for 5 weeks. The adrenal glands of both groups were then subjected to morphological, hormonal and immunohistochemistry analyses. The results showed that DHEAS supplementation led to a significant increase in the accumulation of lipofuscin granules in the adrenal cells. Furthermore, decreases in ERα and ERβ and the enzymes CYP17 and 17βHSD, and an increase in the 5α-reductase enzyme in the adrenal cortex were also observed. The results suggest that DHEAS supplementation has a negative feedback effect on the adrenal cortex, affecting its morphophysiology and, consequently, the gland's functionality. In addition, DHEAS supplementation does not reverse all aspects of the effects of aging on adrenal gland homeostasis.

1. Introduction

The adrenal gland is an important gland in the endocrine system, responsible for many regulatory functions and physiological processes to promote homeostasis in the body. The adrenal cortex consists of three zones with different morphology and functions. The zona glomerulosa (ZG) is responsible for the secretion of mineralocorticoids, the zona fasciculata (ZF) for the synthesis of glucocorticoids, and the zona reticularis (ZR) for producing androgens [1]. These steroid hormones are responsible for regulating the homeostasis of ions in the blood, the mobilization of glucose and lipids, and the control of inflammation and the immune system, in addition to initiating sexual development and sexual secondary characteristics [2].

In children and women, the adrenal gland is one of the main sources of androgens, mainly through the production of dehydroepiandrosterone (DHEA) and its sulphated form, dehydroepiandrosterone sulphate (DHEAS) [3]. Androgens begin to be produced during middle childhood with the differentiation of the ZR, in a process known as adrenarche [4]. DHEA is synthesized in the adrenal cortex from cholesterol, which is converted into pregnenolone. Through the action of cytochrome P450 17A1 (CYP17), pregnenolone is converted into 17a-hydroxypregnenolone and finally into DHEA [5]. DHEA can be reversibly converted into its sulphated form, DHEAS, by the enzyme sulfotransferase 2A1 (SULT2A1), a more stable form of the hormone that is usually found in

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the bloodstream in greater quantities [6]. Meanwhile, in reproductive organs like ovaries and testes, DHEAS can be converted into other more potent androgens, such as androstenediol, androstenedione, and testosterone, by the action of the 3β-hydroxysteroid dehydrogenase (3βHSD) and 17β-hydroxysteroid dehydrogenase (17βHSD) [7]. At the end of the steroidogenic pathway, testosterone can also be converted into dihydrotestosterone by the action of 5α-reductase [8], or into estradiol by the action of aromatase (CYP19) [9].

After adrenarche, DHEA synthesis by the ZR continues to increase, reaching peak production in humans at around the age of 25. Around the age of 30, hormone levels begin to decrease and during aging these levels drop dramatically, resulting in low DHEA levels [10]. This process occurs due to the morphological and enzymatic alterations caused by aging in the adrenal cortex, which confers the adrenopause period [11]. In women, this period is highly significant, given that most of the androgens present in females are produced in the adrenal glands and the decrease in DHEA levels accelerates the menopausal processes, favoring the emergence of features such as atrophy of the skin and vagina, as well as muscle and bone density loss [12].

Due to this hormonal decline that occurs during aging, some women take supplements with exogenous DHEAS. This supplementation leads to some significant results, such as improving the immune system and the skin, as well as increasing sexual libido [13]. Specifically in postmenopausal women, DHEAS supplementation has been shown to restore androgen and estrogen levels, as well as aiding in the restoration of bone density and preventing osteoporosis [14]. In addition to these effects, studies have shown that DHEAS supplementation has positive effects in relation to other diseases, such as diabetes, autoimmune diseases, erectile dysfunction, and breast cancer, as well as helping to increase muscle strength [15]. Moreover, in adrenal insufficiency patients, DHEAS supplementation has been shown to improve lipid metabolism, sexual function, and fertility [16].

Despite this, the effects of DHEA supplementation remain unexplored in the adrenal cortex of aging woman. Therefore, the aim of the current study was to analyze the effects of DHEAS supplementation in the adrenal cortex of aged female Mongolian gerbils. Mongolian gerbils (*Meriones unguiculatus*) were used as an experimental model due to its adrenal morphophysiological similarities with humans and responsiveness during aging [17]. Mice and rats, which are commonly used as experimental models, have differences in adrenal morphophysiology compared to humans. The adrenal glands of mice and rats have only two well-defined zones (ZG and ZF), and these rodents lack some steroidogenic enzymes such as CYP17 and do not produce cortisol and androgens in the adrenal cortex [18]. The Mongolian gerbil, on the other hand, has three well-defined zones of the adrenal cortex and enzymes and hormones similar to humans [17,19].

2. Material and methods

2.1. Animals and experimental design

During the experiment, the animals were kept under controlled temperature (23 °C) and light conditions (12 h light/12 h dark) and provided with water and rodent food *ad libitum*. The procedures were carried out in accordance with National Research Council's Guide for the Care and Use of Laboratory Animals (USA) and National Council of Control and Animal Experimentation (CONCEA, Brazil) regulations and approved by the local Ethics Committee on the Use of Animals (CEUA, IBILCE, UNESP, Brazil), under protocol n° 229/2021.

To perform this study, age virgin females of Mongolian gerbils (18 months old) (Animal Breeding Center of the Institute of Biosciences, Humanities and Exact Sciences (IBILCE) of the São Paulo State University (UNESP), São José do Rio Preto, São Paulo, Brazil) were divided into two experimental groups (n = 5/group): Control group (C), animals received no treatment; dehydroepiandrosterone sulfate (DHEAS) group (DHEAS), animals received subcutaneous injections of Dehydroepiandrosterone Sulfate (60 mg/kg) (sodium salt, 15873, purity ≥ 95 %, Cayman Chemical Company, Ann Arbor, Michigan, USA) diluted in mineral oil once a week for 5 weeks. DHEAS supplementation during aging was based on a previous study [20], adapted to the Mongolian gerbil. At the end of the treatment period, vaginal smears were taken from the female gerbils and analyzed to determine the phase of the estrous cycle. All the females were euthanized during the estrus phase to standardize the hormonal variations resulting from the estrus cycle. The animals were euthanized by anesthesia with isoflurane (3 %), followed by decapitation for blood collection. The collected blood was centrifuged, and the serum was stored at −80 °C for hormonal analysis. The adrenal glands were dissected, weighed, and fixed in paraformaldehyde (4 %) for 24 h.

2.2. Hormonal analysis

The levels of circulating DHEAS were measured in the serum of 5 animals/group and quantified using a commercial kit. The assay was performed in duplicate according to the commercial kit DHEAS ELISA (RE52181, Lot EDS136, 0.1–10 μg/mL, IBL International, Hamburg, DE, Germany), following the manufacturer's instructions. The analyses were performed in a Spectra Max Plus 384 microplate reader (Molecular Devices, San Jose, CA, USA).

2.3. Morphological analysis

The paraformaldehyde-fixed adrenal glands were subjected to histological processing, embedded in paraffin Histosec® (Merck, Darmstadt, Hesse, Germany), and sectioned at a thickness of 5-μm to obtain histological sections. The histological sections were stained with hematoxylin-eosin (HE) for morphometric analysis and with Gömöri-Halmi trichrome for analysis of the accumulation of lipofuscin granules. For the morphometric analyses, the HE-stained slides were analyzed under a light microscope using the slide scanner system (VS120–S5, Olympus, Tokyo, Japan) at 400x magnification. Morphometric analysis was performed using QuPath® software (Version 0.4.2) and 35 measurements were taken of each zone per animal, totaling 210 measurements per group. To quantify the lipofuscin granules, 15 photos of each zone per animal were taken from the slides stained with Gomori-Halmi trichrome. The percentage of areas containing lipofuscin granules was quantified using Image J software (Version 1.8.0, Bethesda, Maryland, USA).

2.4. Immunohistochemistry analysis

Immunohistochemistry analyses were carried out to quantify cell proliferation, cell death, hormone receptors, and steroidogenic enzymes in the adrenal cortex. For this analysis, slides with histological sections were deparaffinized and rehydrated. Antigenic recovery was then carried out using 10 mM sodium citrate buffer (pH 6) or 10 mM Tris-EDTA buffer (pH 9) at 98 °C for 45 min. Endogenous peroxidase was blocked with two 20-min baths of hydrogen peroxide (10 %) in methanol. Nonspecific proteins were blocked with skimmed milk powder (5 %) for 1 h. Between each step of the protocol, the slides were washed with PBS + Tween. The histological sections were incubated with primary antibodies: PHH3, active/cleaved caspase-3, AR, ERα, ERβ, 17BHSD, 3BHSD 5α-Red, CYP17, and CYP19 overnight at 4 °C. More information on the primary antibodies and buffers used is shown in Table 1. Immunoreactions for the detection of Caspase-3, CYP17, 3BHSD, 5α-Red, 17BHSD, and CYP19 were incubated with a polymer Refine Red Detection kit (REF. DS9390, Leica Biosystems Newcastle Ltd.), and revealed using an alkaline phosphatase kit (Vulcan Fast Red Chromogen Kit 2, REFFR8055, Biocare Medical, California, USA). Immunoreactions for the detection of PHH3, AR, Erα, and ERβ were incubated with post-primary antibody and polymer (Novolink™ Polymer Detection System 1, Leica Biosystems Newcastle Ltd.) for 60 min at room temperature.

Table 1
Description of the antibodies and protocols used in immunohistochemistry.

	Antigen retrieval	Primary antibody reference	Primary antibody dilution
PHH3	10 mM sodium citrate buffer pH 6	Rabbit polyclonal, Ser10, 9701, Cell Signaling, Danvers, Massachusetts, USA	1:75 in BSA 1 %
Caspase-3	10 mM TRIS-EDTA buffer pH 9	Rabbit polyclonal, NB100-56113, Novus Biological, Denver, Colorado, USA	1:50 in BSA 1 %
AR	10 mM sodium citrate buffer pH 6	Mouse monoclonal, sc-7305, Santa Cruz Biotechnology, Dallas, Texas, USA	1:50 in BSA 1 %
ERα	10 mM sodium citrate buffer pH 6	Mouse monoclonal, sc-8005, Santa Cruz Biotechnology, Dallas, Texas, USA	1:50 in BSA 1 %
ERβ	10 mM sodium citrate buffer pH 6	Rabbit polyclonal, PA1-310B, Invitrogen, Thermo Fisher, Waltham, Massachusetts, USA	1:50 in BSA 1 %
17BHSD	10 mM TRIS-EDTA buffer pH 9	Rabbit polyclonal, sc-32872, Santa Cruz Biotechnology, Dallas, Texas, USA	1: 50 in BSA 1 %
3BHSD	10 mM TRIS-EDTA buffer pH 9	Rabbit polyclonal, E-AB-15114, Elabscience, Houston, Texas, USA	1:50 in BSA 1 %
5α-Red	10 mM TRIS-EDTA buffer pH 9	Mouse monoclonal, sc-293232, Santa Cruz Biotechnology, Dallas, Texas, USA	1:50 in BSA 1 %
CYP17	10 mM TRIS-EDTA buffer pH 9	Rabbit polyclonal, E-AB-11124, Elabscience, Houston, Texas, USA	1:50 in BSA 1 %
CYP19	10 mM TRIS-EDTA buffer pH 9	Mouse monoclonal, sc-74176, Santa Cruz Biotechnology, Dallas, Texas, USA	1:50 in BSA 1 %

Detection of positive staining was performed with 3,3-diaminobenzidine tetrahydrochloride (DAB) (Novolink™ DAB, RE7270-CE, Leica Biosystems, Newcastle Ltd.). Finally, the slides were counterstained with hematoxylin.

The immunohistochemical analyses were quantified using QuPath® software (Version 0.4.2, Edinburg, Scotland) [21]. The software identifies the immunohistochemistry-positive cells in each zone of the adrenal cortex by color. It also quantifies the total number of cells present in the region and calculates the percentage of positive cells (n = 5/group) [19].

2.5. Statistical analysis

The data were subjected to the Kolmogorov-Smirnov normality test. The parametric data were then subjected to the unpaired *t*-test, and non-parametric data were analyzed by the Mann–Whitney test. Data are presented as the mean and standard deviation and the significance level adopted was 5 % ($p \leq 0.05$). Statistical analyses were performed in GraphPad Prism software (Version 8.0.1, Boston, Massachusetts, USA).

3. Results

3.1. Hormonal and biometric data

Table 2 presents the hormonal quantification of DHEAS in the serum of female Mongolian gerbils. As expected, there was an increase in serum DHEAS levels in the treated animals compared to the control group (P = 0.0171). The biometric data, also presented in Table 2, showed no alterations in the body weight of the animals or in the absolute and relative weights of the adrenal glands (Table 2).

3.2. Morphological data

The morphological analyses are represented in Fig. 1. DHEAS supplementation did not promote alterations in the thickness of the adrenal cortex zones (Fig. 1 B, C, D). Halmi staining showed an increase in the accumulation of lipofuscin granules in the cells of all zones of the adrenal cortex in the animals treated with DHEAS (P = 0.0051, P = 0.0079 and P = 0.004) (Fig. 1 E, F, G).

Table 2
Hormonal and biometric data. The data were expressed as mean followed by standard deviation[1].

	C	DHEAS
Hormonal data (n = 5/group)		
DHEAS (μg/ml)	0.12 ± 0.01[a]	0.21 ± 0.07[b]
Biometric data (n = 5/group)		
Body weight (g)	76.00 ± 7.02	77.71 ± 9.69
Right adrenal weight (g)	0.023 ± 0.007	0.029 ± 0.010
Left adrenal weight (g)	0.027 ± 0.006	0.029 ± 0.008
Relative right adrenal weight ($\times 10^{-3}$)	0.31 ± 0.11	0.39 ± 0.16
Relative left adrenal weight ($\times 10^{-3}$)	0.35 ± 0.79	0.37 ± 0.12

[1] Parametric data were subjected to the unpaired T-test and non-parametric data were subjected to the Mann-Whitney test. a ≠ b. C: Control group; DHEAS: Dehydroepiandrosterone sulfate.

3.3. Cell proliferation and cell death

Fig. 2 presents the immunohistochemistry analyses for quantifying cell proliferation and death (Fig. 2A). No alterations were observed in the rates of proliferation (Fig. 2 B, C, D) and cell death (Fig. 2 E, F, G) in any of the adrenal cortex zones after treatment with DHEAS.

3.4. Steroid hormone receptors

Fig. 3 presents the immunohistochemistry analyses for the identification of steroid hormone receptors (Fig. 3 A). Treatment with DHEAS did not alter AR in the adrenal cortex (Fig. 3 B, C, D). Regarding ERα, it was possible to observe a decrease in ZG and ZR in the animals supplemented with DHEAS (P = 0.0003 and P = 0.0005, respectively) (Fig. 3 E, F, G). There was also a decrease in ERβ in the DHEAS-treated animals, but this decrease only occurred in the ZF of the adrenal cortex (P = 0.0036) (Fig. 3 H, I, J).

3.5. Steroidogenic enzymes

Fig. 4 presents the immunohistochemistry analyses to identify the key steroidogenic enzymes in the adrenal cortex (Fig. 4 A). The CYP17 enzyme decreased in the DHEAS group in the ZF and ZR (P = 0.0116 and P = 0.0202, respectively) (Fig. 4 B, C, D), and the 3βHSD enzyme showed no statistical differences (Fig. 4 E, F, G), and the 17βHSD enzyme also decreased in the ZF and ZR of the animals in the DHEAS group (P = 0.0359 and P = 0.0011, respectively) (Fig. 4 H, I, J). The 5α-reductase enzyme increased in the ZG and ZF of the animals supplemented with DHEAS (P = 0.0390 and P = 0.0355, respectively) (Fig. 4 K, L, M). Finally, the CYP19 enzyme showed no statistical differences (Fig. 4 N, O, P).

Fig. 5 summarizes the main alterations found in the adrenal cortex, promoted by DHEAS supplementation. In addition, Fig. 5 presents the alterations in the androgen and estrogen production pathways in the adrenal cortex.

4. Discussion

The main results demonstrated the effect of DHEAS supplementation during aging on the morphophysiology of the adrenal cortex. This supplementation in aging females promotes an alteration in ERs, imbalance in key steroidogenic enzymes, such as CYP17, 17βHSD, and 5α-reductase, in addition to an accumulation of lipofuscin granules. The present study is one of the first to evaluate the adrenal glands of female

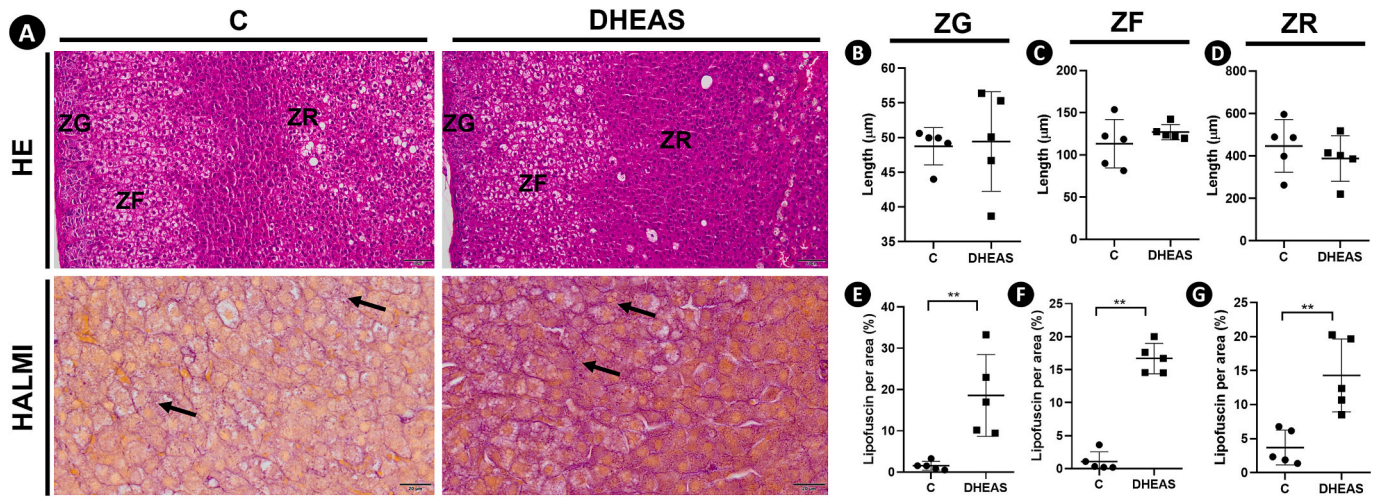

Fig. 1. Effects of DHEAS on the morphology and accumulation of lipofuscin granules in the adrenal cortex. Photomicrographs of the adrenal cortex stained with hematoxylin and eosin (HE) and Gomori-Halmi trichrome (A). Mean and standard deviation of the thickness of the zona glomerulosa (B), zona fasciculata (C), and zona reticularis (D). Mean and standard deviation of lipofuscin quantification (E-G). Arrows indicate lipofuscin granules. Control group (C) and treated group (DHEAS). Asterisks represent statistically significant differences between the groups (**P < 0.01). Zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR).

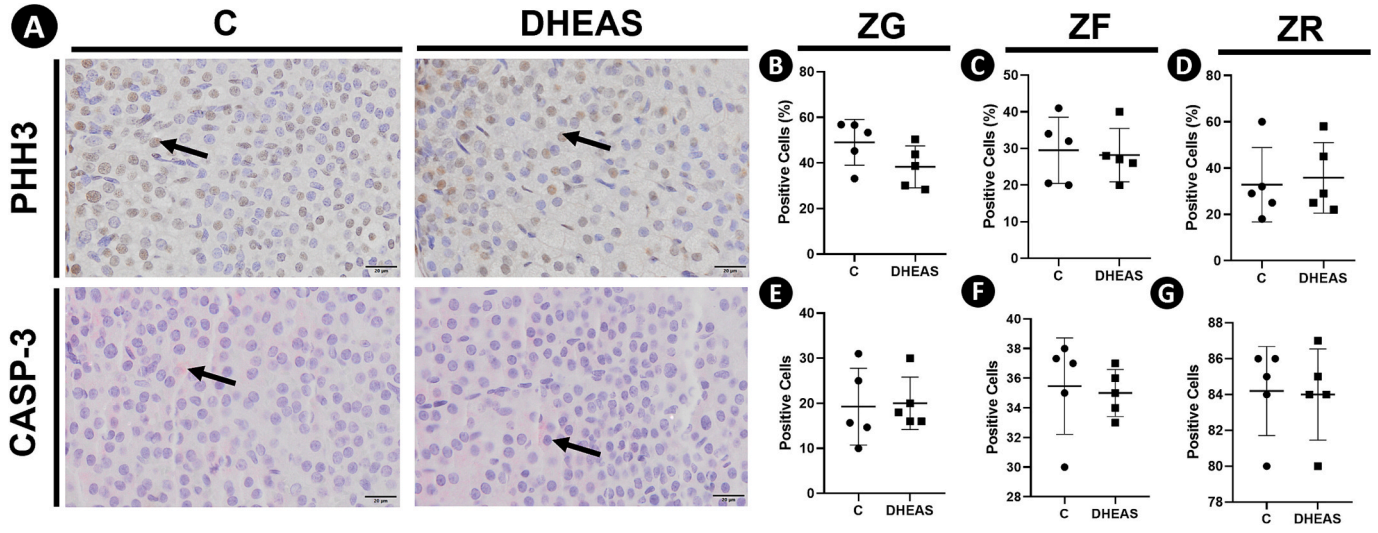

Fig. 2. Effects of DHEAS on proliferation and cell death in the adrenal cortex. Photomicrographs of immunohistochemistry for the identification of Phospho-histone H3 (PHH3) and Caspase-3 (A). Quantification of PHH3 (B-D) and Caspase-3 (E-G). Arrows indicate positive cells for immunolabeling. Control group (C) and treated group (DHEAS). Zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR).

Mongolian gerbils. The morphology of the three areas of the cortex is similar to primates and the gland expresses steroidogenic enzymes similarly to males [19]. The gerbil also shares physiological aspects of glandular aging with primates, such as dysregulation of the lipid metabolism and steroidogenic pathways [22].

Mongolian gerbil females tend to present naturally decreasing DHEAS levels during aging [23]. This occurs similarly in humans, with a decrease in the amounts of DHEA and DHEAS, due to a decrease in adrenal function and its steroidogenic enzymes, in a process called adrenopause [10,24]. The hormonal supplementation with exogenous DHEAS used in the current experiment was able to reverse this natural decrease in the hormone during aging, causing a significant increase in DHEAS in the females. This supplementation may delay or prevent the effects of adrenopause and, consequently, affect menopause, however, the adverse effects of this supplementation are not yet well known [13].

During aging, the adrenal cortex can undergo alterations in its morphology and in hormone production, especially of androgens [25]. Although the DHEAS supplementation did not promote alterations in the morphology of the cortical zones, an increase was observed in the accumulation of lipofuscin granules in the cells of the adrenal cortex. Lipofuscin granules are an indicator of aging and cellular senescence [26]. The adrenal gland tends to naturally accumulate lipofuscin granules during aging and this accumulation is related to the decline in androgen production in the adrenal gland [27]. In addition, the exacerbated accumulation of lipofuscin in the adrenal gland can accelerate the glandular aging process and cause mutations, leading to the formation of tumors, such as in Cushing's syndrome [28].

The adrenal gland exhibits sexual dimorphism in the rates of cell proliferation and death [29]. This is because the rates of cell renewal in the adrenal cortex are influenced by the action of androgens such as testosterone and dihydrotestosterone [30]. Male adrenal cell renewal responds to castration or testosterone supplementation [19]. DHEAS supplementation in females, on the other hand, did not alter the rates of proliferation and cell death in the adrenal cortex, which was reflected in

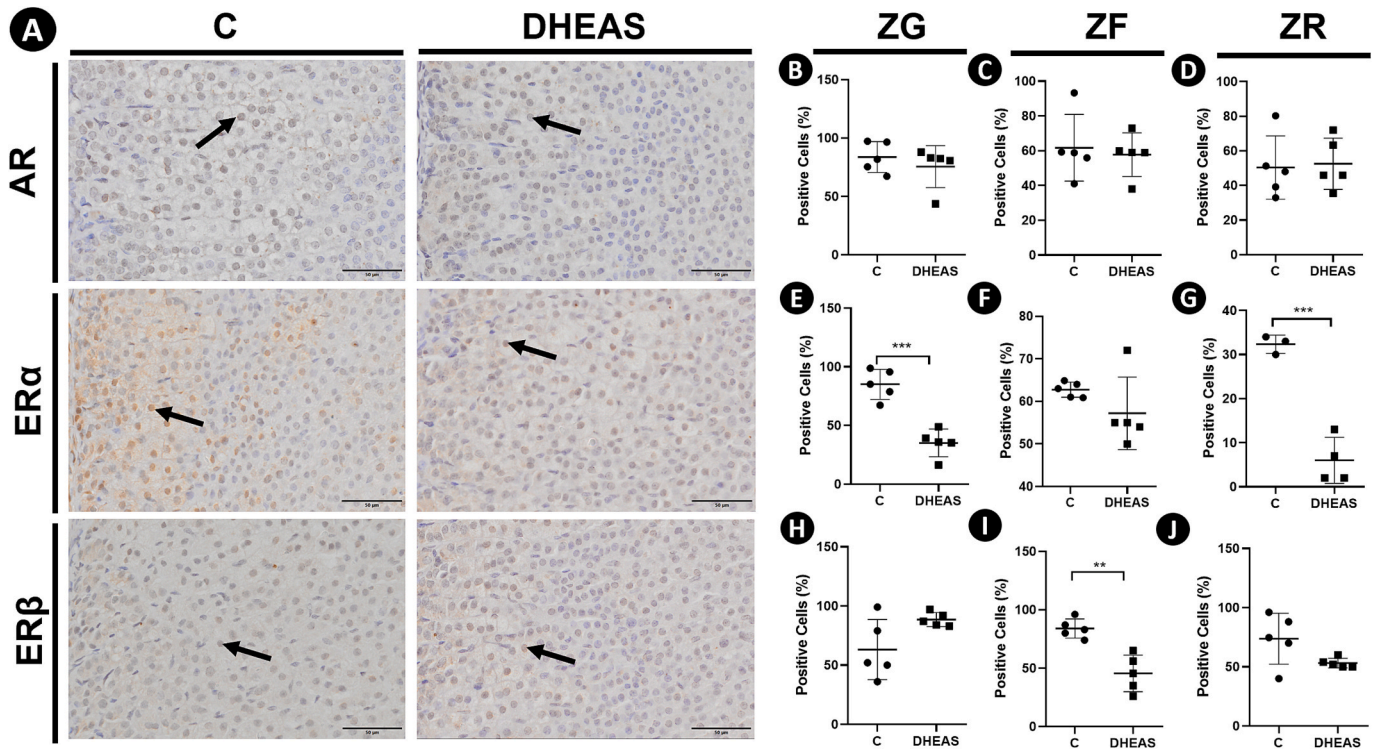

Fig. 3. Effects of DHEAS on hormone receptors in the adrenal cortex. Photomicrographs of immunohistochemistry for the identification of androgen receptors (AR), estrogen receptors alpha (ERα), and estrogen receptors beta (ERβ) (A). Quantification of AR (B-D), ERα (E-G), and ERβ (H-J). Arrows indicate positive cells for immunolabeling. Control group (C) and treated group (DHEAS). Asterisks represent statistically significant differences between the groups (**P < 0.01; *** P < 0.001). Zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR).

the biometric data through the adrenal weight. This is a particular feature of the adrenal glands of female gerbils, as older males showed an increase in cell death as a characteristic of aging [17].

Steroid hormone receptors play an important role in regulating the adrenal cortex. The AR plays a mediating role between adrenal hormones and regulation of the morphophysiology of the adrenal cortex [31]. DHEAS supplementation did not alter the frequency of AR in the adrenal cortex. This may be related to the fact that proliferation rates are not altered, given that AR also acts to regulate this process [32]. However, when estrogen receptors (ERs) were evaluated, it was possible to observe that DHEAS promoted a decrease in these receptors. ERα plays an important role in regulating adrenarche and in the production of DHEA by the adrenal glands, and tends to increase with age [33]. Androgen modulation, such as orchiectomy, was able to alter adrenal ERα levels [34]. Thus, DHEAS supplementation may have triggered negative feedback in the gland, in which the increase in exogenous hormone promoted a decrease in ERα to stimulate a decrease in endogenous glandular production and, consequently, to balance hormone levels. The same occurred with ERβ, which decreased in the ZF after the treatment and which also plays a role in regulating the production of other adrenal hormones, such as aldosterone and glucocorticoids [35,36]. Therefore, supplementation with DHEAS promotes deregulation of ERs which can interfere not only in the production of adrenal androgens but also in other steroidogenesis pathways of the gland.

The steroid hormones produced in the adrenal glands from cholesterol are synthesized by the steroidogenic enzymes present in the adrenocortical cells [2]. CYP17 is a key enzyme in the production of DHEA, converting pregnenolone into 17α-hydroxypregnenolone and then converting it into DHEA [37]. Another enzyme present in the adrenal cortex is 17βHSD, which converts DHEA into other androgens, such as androstenediol and testosterone [38]. Both enzymes decreased after DHEAS supplementation. This decrease in enzymes may be a way of compensating for the androgen production in the adrenal glands following supplementation, thus balancing hormone levels. A similar phenomenon occurs in the testicles when there is testosterone supplementation, which causes negative feedback on the hypothalamic-pituitary–gonadal (HPG) axis and results in a decrease in testicular steroidogenic enzymes and inhibition of endogenous testosterone production [39]. Thus, supplementation with DHEAS can cause inhibition of these enzymes which can compromise the functioning of the gland.

In the steroidogenic pathway, another steroidogenic enzyme present in the adrenal glands is 5α-reductase, an enzyme responsible for converting testosterone into the more potent androgen, 5α-dihydrotestosterone, and present in various organs, such as the liver, prostate, and skin [5]. The increase in 5α-reductase in the adrenal cortex caused by DHEAS may be a response to greater androgen availability in the body, which converts these androgens into dihydrotestosterone, that has functions in other tissues, such as increasing muscle mass and bone density [40]. The increase in 5α-reductase has also been observed in the ovaries of Mongolian gerbils after exposure to BPA, promoting hormonal alterations in the female's reproduction axis, indicating that this enzyme is sensitive to endocrine disruption [41]. These enzymatic alterations show that DHEAS supplementation promotes a feedback effect on the steroidogenesis process in the adrenal cortex, reinforcing our hypothesis. These alterations may have an impact on the production and availability of other steroid hormones, affecting the body's homeostasis in older females.

In addition to the parameters evaluated in this study, it would be interesting to evaluate the effects of DHEAS supplementation on the production of other steroid hormones such as testosterone and estrogen. Furthermore, exploring alternative regulatory pathways in the adrenal cortex, such as ZiP9 and GPER receptors, promises to offer novel insights to the subject and are of great interest for future research. However, it is important to note that the absence of these analyses does not detract from the objective of the study, which is to evaluate the effects of DHEAS

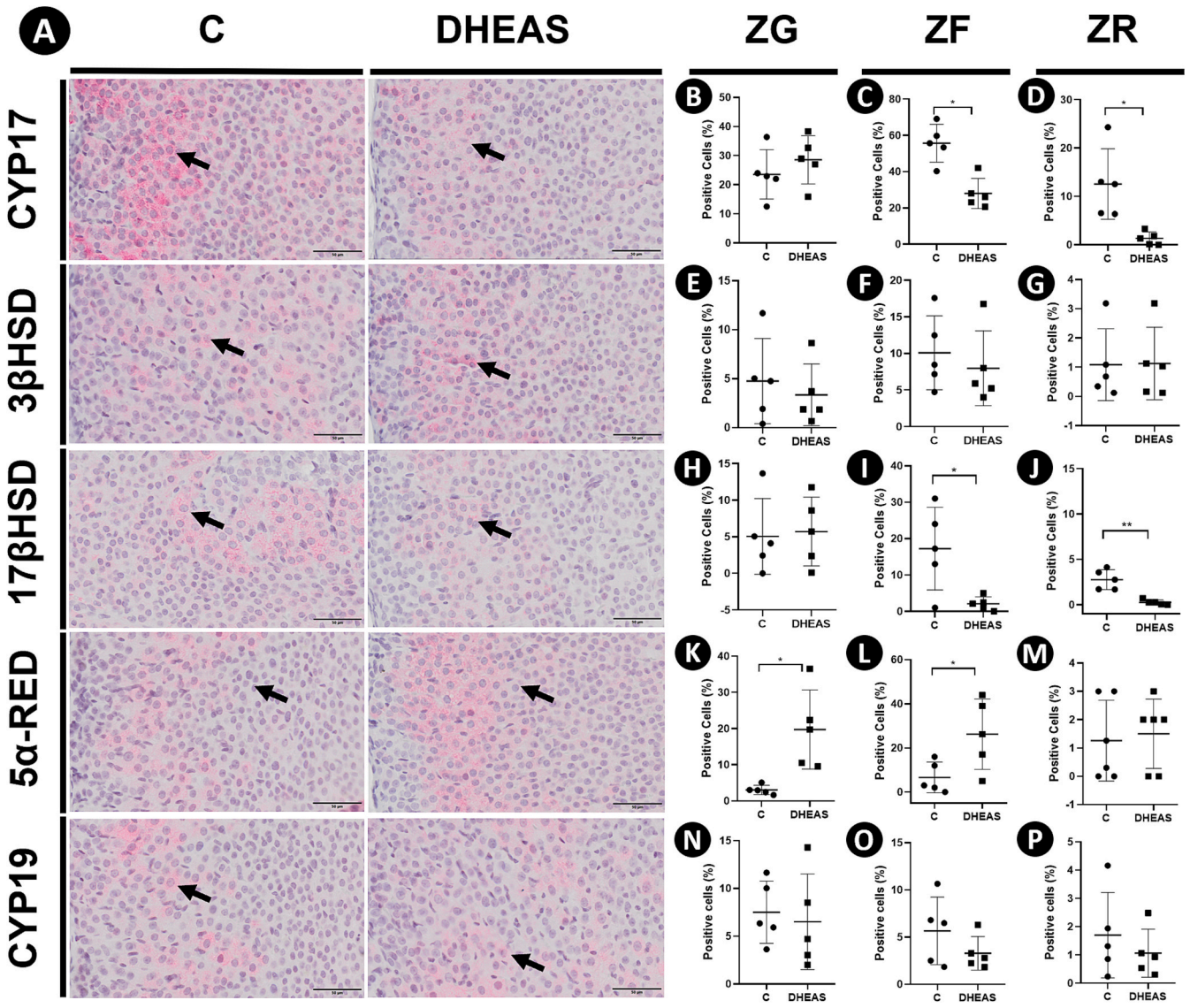

Fig. 4. Effects of DHEAS on steroidogenic enzymes in the adrenal cortex. Photomicrographs of immunohistochemistry to identify CYP17 (B-D), 3βHSD (E-G), 17βHSD (H-J), 5α-reductase (K-M), and CYP19 (N—P) enzymes. Arrows indicate positive cells for immunolabeling. Control group (C) and treated group (DHEAS). Asterisks represent statistically significant differences between the groups (**P < 0.01; *** P < 0.001). Zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR).

on the morphophysiology of the adrenal cortex, with a particular focus on the main enzymes and regulators of the adrenal cortex.

5. Conclusion

The results indicate that DHEAS supplementation does not reverse all aspects of the effects of aging on adrenal gland homeostasis. DHEAS supplementation during aging prevented the natural decrease in the DHEAS levels, delaying the adrenopause process. However, this excess of exogenous hormone promoted a negative feedback effect on the adrenal cortex, altering the frequency of estrogen receptors and steroidogenic enzymes, and increasing the accumulation of lipofuscin granules. These alterations may compromise the morphophysiology of the adrenal gland in the long term and, consequently, have an impact on their function.

CRediT authorship contribution statement

Carolina Marques Bedolo: Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Isabella Carolina Cozim:** Validation, Methodology, Formal analysis. **Vitor Grigio:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Gabriel Ribeiro Bernussi:** Software, Methodology, Formal analysis. **Stella Bicalho Silva:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Luiz Henrique Alves Guerra:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Silvana Gisele Pegorin de Campos:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Patrícia Simone Leite Vilamaior:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Ellen Cristina Rivas Leonel:** Writing – review & editing, Validation, Supervision, Conceptualization. **Sebastião Roberto Taboga:** Writing – review & editing, Validation, Supervision, Resources, Data curation, Conceptualization.

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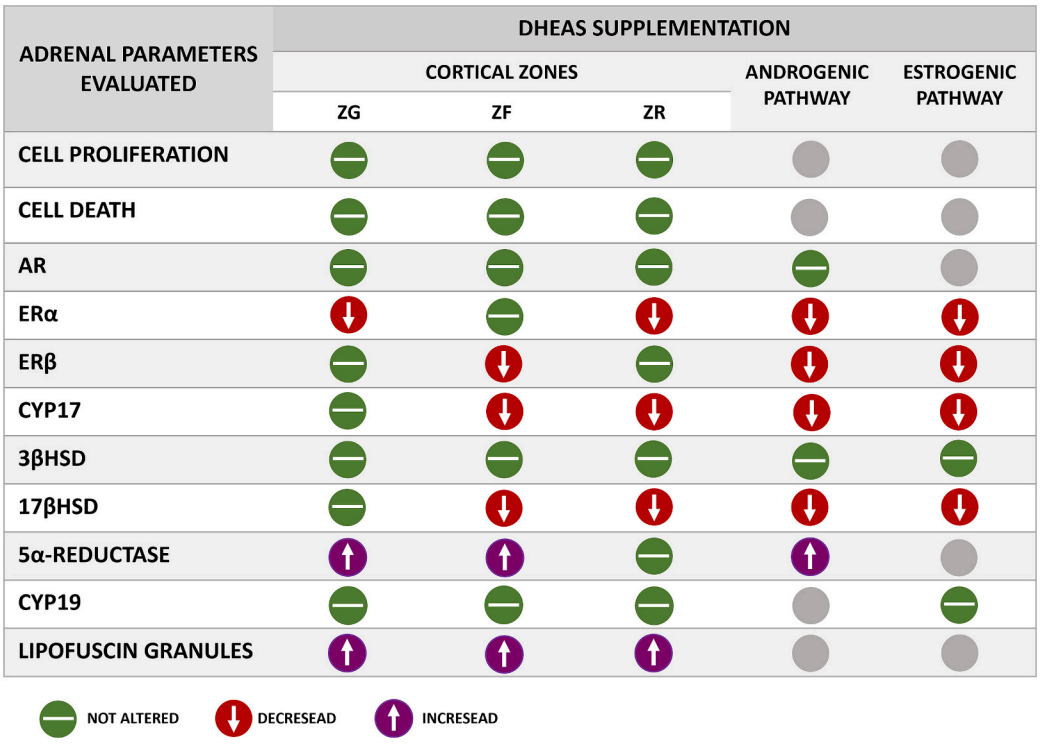

Fig. 5. Summary of the main alterations promoted by DHEAS supplementation in the adrenal cortex. Zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Glossary

AR: androgen receptor
C: Control group
CYP17: cytochrome P450 17A1
CYP19: cytochrome P450 19A1
DAB: 3,3-diaminobenzidine tetrachlorhydrate
DHEA: dehydroepiandrosterone
DHEAS: dehydroepiandrosterone sulfate
ERα: estrogen receptor alpha
ERβ: estrogen receptor beta
ERs: estrogen receptors
HE: hematoxylin and eosin
PHH3: phospho-histone H3
SULT2A1: sulfotransferase 2A1
TBSt: tris-buffered saline with tween
ZF: zona fasciculate
ZG: zona glomerulosa
ZR: zona reticularis
5α-Red: 5α-reductase
17βHSD: 17β-hydroxysteroid dehydrogenase
3βHSD: 3β-hydroxysteroid dehydrogenase