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Avaliação morfofuncional do córtex adrenal em machos de gerbilo da Mongólia durante o envelhecimento

São José do Rio Preto

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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RESUMO

É conhecido que o processo de envelhecimento ocasiona em machos um desequilíbrio hormonal devido à redução gradual da síntese de testosterona pelos testículos, que afeta a maioria dos órgãos sensíveis a hormônios e dentre eles a adrenal, que é responsável pela síntese e secreção de hormônios esteroides como mineralocorticoides, glicocorticoides e andrógenos. Estudos anteriores reconhecem o importante papel da adrenal na regulação do sistema reprodutor e também é conhecido, por meio de estudos com castração, que esta é sensível à redução de andrógenos, no entanto, há pouco entendimento do papel dessa glândula no processo de andropausa e há uma dificuldade em encontrar modelos experimentais representativos, uma vez que há muitas diferenças morfofuncionais entre as adrenais dos roedores e primatas. As adrenais dos gerbilos da Mongólia têm características ultraestruturais peculiares que sugerem uma maior similaridade à dos primatas. Apesar de serem modelos experimentais utilizados em estudos de desregulação endócrina e do sistema reprodutor, há poucos estudos sobre a fisiologia do córtex adrenal dessa espécie. Considerando essa afirmação, esse estudo descreveu pela primeira vez a morfofisiologia da adrenal de gerbilos adultos e durante o envelhecimento, trazendo resultados que demonstram similaridades do córtex adrenal dessa espécie com o humano e de outros primatas, desde a morfologia à expressão de enzimas da biossíntese de cortisol e andrógenos e de receptores de andrógeno e estrógeno. Durante o envelhecimento foi constatada uma hipertrofia do córtex adrenal por meio do aumento do peso relativo e absoluto da glândula, assim como no aumento da espessura das zonas corticais e presença de células proliferativas, evidenciando assim uma possível resposta dessa glândula ao envelhecimento em decorrência do desequilíbrio hormonal. Na análise morfológica foi observada maior quantidade de tecido conjuntivo nos animais de 15meses e um acúmulo de lipofuscina na zona reticular nos grupos de 12 e 15meses, assim como regiões com células hipertróficas com grande quantidade de conteúdo lipídico, características que são sinais de senescência decorrentes do decréscimo do metabolismo celular. Tais resultados levam à conclusão de que nas idades de 12e 15meses o córtex adrenal dos gerbilos apresenta sinais de senescência apesar de também demonstrar uma hipertrofia, achados similares aos encontrados em primatas, indicando que essa espécie pode ser um modelo experimental adequado para estudo da adrenal.

Palavras-chave: Adrenal. Gerbilo. Envelhecimento.

ABSTRACT

The aging process in males is known by occasioning a hormonal imbalance due to the gradual reduction in the testicular testosterone synthesis, which affects most of the organs sensitive to this hormone and, among them the, adrenal glands which its cortex is responsible to synthesize and secrete steroidogenic hormones as mineralocorticoids, glucocorticoids, and androgens. Existing research recognizes the significant role played by this gland in the regulation of the reproductive system; it is also known that it is sensitive to the reduction of testosterone caused by castration, although there is still very little scientific understanding of its role in the andropause process. Moreover, Mongolian gerbils are experimental models for studies in reproductive biology and endocrine deregulation, their adrenal glands present a peculiar structure and suggest having more similar morphophysiology to the primates than others rodents, although there have been few investigations about the gerbils' adrenocortical physiology. Considering this, this study has described the morphophysiology of this gland in gerbil for the first time and showed particular similarities between the adrenal cortex of *M. unguiculatus* and primates, since the morphology, to the expression of enzymes of cortisol and androgens biosynthesis and androgen and estrogen receptors. During aging was an hypertrophy in the adrenal cortex, based on the gradual increase in adrenal absolute weight of the adrenals in the older groups and the relative weight presents an increase in the older group (15months), as well as expression of proliferation markers highlighting the role of this gland at this stage of aging, when the sexual hormones are decreasing, as well as the morphometry has shown a larger length of all cortical areas. General morphological analysis showed in older groups larger connective tissue between cortex and medulla and increase of collagen fibers and in the reticular zone were seen lipofuscin accumulation in animal with the age of 12 and 15 months, as well as regions with larger cells and clear cytoplasm, this may reflect the decrease in the metabolic activities of the cell, due to the senescence process, leading us to conclude that at the age of 12 and 15 months the adrenal presents signs of senescence, although it shows a hypertrophy demonstrating a probable compensatory function to the low cell metabolism and to the androgens decrease, this finding is similar to the research in primates making of it a possible experimental model for adrenal diseases studies.

Key-words: Adrenal. Gerbil. Aging.

LISTA DE ABREVIAÇÕES

3BHSD/ HSD3B2 3β desoxidesidrogenase

AC Adenilato ciclase

ACTH Hormônio adeno-corticotrófico

CASH Hormônio estimulador de andrógeno cortical

CRH Hormônio liberador de corticotrofina

CYB5 Citocromo B5

CYP/ CYP450 Citocromo p450

CYP17 Citocromo p450 17α hidroxilase/liase

CYP11B2 Citocromo p450 aldosterona sintetase

DHEA Dehidroepiandrosterona

DHT Diidrotestosterona

Dz Zona Definitiva

Fz Zona Fetal

LOH Late Onset Hypogonadism

PADAM (Partial) Androgen Decline in the Aging Male

PKA Fosfocinase A

POMC Pré-pró-opiomelanocortina

SDHEA Sulfato de Dehidroepiandrosterona

SHH Sonic Hedgehog

T Testosterona

TDS Testosterone Deficiency Syndrome

TNF-α Fator de Necrose Tumoral alfa

ZF Zona Fasciculada

ZG Zona Glomerulosa

ZR Zona Reticular

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1 INTRODUÇÃO

O sistema endócrino sofre diversas alterações durante o envelhecimento, principalmente devido à redução da produção dos hormônios sexuais. Nas fêmeas esse é um fenômeno bastante estudado, devido à cessação da ovulação e, consequentemente da produção de estrógenos, conhecida como menopausa. Em machos, porém, essa redução é gradativa e a síntese dos andrógenos pelas gônadas não cessa completamente, no entanto, conhece-se relativamente pouco sobre esse processo, denominado andropausa e atualmente também chamada de Síndrome de deficiência androgênica, PADAM (Partial Androgen Decline in the Aging Male), LOH (Late Onset Hypogonadism), TDS (Testosterone Deficiency Syndrome) (PARK; AHN; MOON, 2019; VANCE, 2003; YADAV et al., 2019) .

A função desempenhada pela glândula adrenal tem um papel importante no processo de envelhecimento devido, principalmente, à secreção dos glicocorticoides e andrógenos, que sabe-se ter seus níveis séricos alterados durante a andropausa e têm correlação com os níveis de testosterona circulante (AJDŽANOVIĆ; MILOŠEVIĆ; SPASOJEVIĆ, 2012; BAULIEU et al., 2000; CUSAN et al., 1997).

O córtex adrenal, em especial a Zona Reticular (ZR) tem papel na produção dos andrógenos (também chamados de andrógenos C9 ou precursores de andrógenos), sendo o maior sítio de síntese desses hormônios em fêmeas e o segundo maior em machos, uma vez que nestes a maior produção de andrógenos é testicular (KRONENBERG et al., 2016). Os andrógenos adrenais atuam no sistema reprodutor, no desenvolvimento das características sexuais secundárias e no comportamento (KRONENBERG et al., 2016; SOMA et al., 2008).

Considerando que a testosterona em machos provém em 95% dos testículos e os 5% restantes, majoritariamente da adrenal, espera-se que essa glândula tenha um papel significativo durante a ausência ou redução dos andrógenos testiculares, uma vez que, em ratos e algumas espécies de gerbilo, sabe-se que essa glândula tem sua fisiologia alterada e a ZR apresenta hipertrofia após a orquiectomia (AJDŽANOVIĆ et al., 2016; BENMOULOUD et al., 2014; ZATRA et al., 2018). Como no envelhecimento também há uma redução gradual dos andrógenos testiculares, acredita-se que o córtex adrenal também está relacionado e apresenta respostas a esse processo (LOIS et al., 2014).

Estudos em ratos como modelos de andropausa utilizam animais castrados para simular a redução de andrógenos decorrentes do envelhecimento (AJDŽANOVIĆ et al., 2016; MILOŠEVIĆ et al., 2018). No entanto, sabe-se que a andropausa é um processo gradual,

variável entre a população masculina e ocasiona uma redução nos andrógenos testiculares, porém não tão drástica ou absoluta, como na castração (VANCE, 2003). Logo, para melhor compreender esse processo são necessários modelos experimentais mais representativos.

1.1 Morfofisiologia da adrenal

As adrenais são glândulas endócrinas com formato triangular e achatado, localizadas acima dos rins (KRONENBERG et al., 2016). Elas são recobertas por uma cápsula conjuntiva e divididas em duas regiões principais: medula e córtex, que se localizam, respectivamente, na porção central e na porção periférica da glândula. Essas regiões possuem origens embrionárias distintas e, por essa razão, podem ser consideradas como órgãos distintos por alguns autores. A medula tem origem da crista neural, ou seja, neuroectodérmica, enquanto o córtex tem origem mesodérmica (Junqueira & Carneiro 2013). Elas também se diferenciam quanto à secreção, pois as células da medula secretam catecolaminas, como adrenalina e noradrenalina, enquanto as do córtex secretam hormônios esteroides (KRONENBERG et al., 2016).

O córtex adrenal, assim como testículos e ovários, tem origem do primórdio adrenoganadal (AGP), este é formado no epitélio celômico em torno de 28-30 dias pós concepção (dpc) em humanos, e expressa um receptor importante para a formação e esteroidogênese da adrenal, o fator estereidogênico 1 (SF1) (XING et al., 2015). As células progenitoras do córtex migram dorsomedialmente e células da crista neural, que originarão as células cromafins da medula, migram e se misturam a elas (PIHLAJOKI et al., 2015). Até o 52 dpc há o completo encapsulamento do córtex em desenvolvimento, pela formação de um tecido fibroso ao redor do córtex em desenvolvimento (KEEGAN; HAMMER, 2002).

Após o encapsulamento há um crescimento do córtex, que apresenta duas regiões diferentes: a zona fetal (Fz), com células maiores e mais eosinófilas e a zona definitiva (Dz), com poucas células pequenas e basófilas (PIHLAJOKI et al., 2015). A adrenal fetal é grande, chegando a ter tamanho similar ao rim, devido a Fz que ocupa maior parte do córtex e sintetiza grandes quantidades de DHEA e SDHEA, hormônios importantes para a manutenção da gestação, uma vez que são convertidos em estrógenos pela placenta (XING et al., 2015).

A zona fetal involui por apoptose logo após o nascimento e a zona definitiva se diferencia nas três zonas corticais: Zona glomerulosa (ZG), Zona Fasciculada (ZF) e Zona

Reticular (ZR) (MOROHASHI; ZUBAIR, 2011). Em camundongos o equivalente a Fz é a chamada zona-X, esta involui ainda no período fetal e uma camada remanescente permanece até o início da puberdade em machos ou até a primeira gestação, em fêmeas (MOROHASHI; ZUBAIR, 2011).

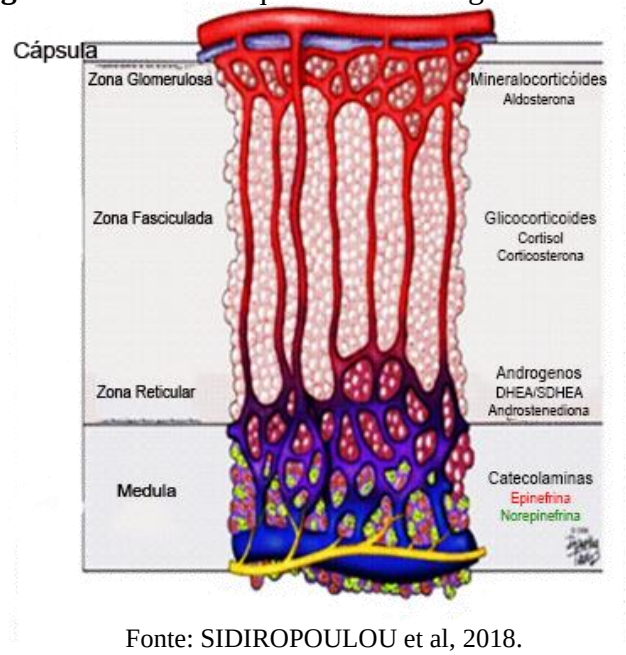
As três zonas corticais, Zona glomerulosa (ZG), Zona Fasciculada (ZF) e Zona Reticular (ZR), são diferenciadas entre si pelo tipo de secreção e pela morfologia **(Figura 1)**. Quanto à secreção, a ZG secreta mineralocorticoides, principalmente, aldosterona, que atua na reabsorção de sódio; enquanto a ZF secreta glicocorticoides como cortisol, cortisona e corticosterona, que são importantes no metabolismo de glicose e de ácidos graxos, na homeostase e na adaptação ao estresse crônico; e a ZR secreta, principalmente, hormônios andrógenos: Dehidroepiandrosterona (DHEA), sulfato de Dehidroepiandrosterona (SDHEA) e androstenediona, mas também pode secretar glicocorticoides (Junqueira & Carneiro 2013; Gallo-Payet & Battista 2014; Kronenberg *et al.* 2016).

A diferença funcional dessas zonas pôde ser comprovada em estudos anteriores em primatas humanos e não humanos pela expressão de diferentes enzimas esteroidogênicas em cada uma delas (Gallo-Payet & Battista 2014). A expressão de P450- aldosterona sintetase (*CYP11B2)*, responsável pela síntese de aldosterona, foi encontrada somente na ZG, enquanto na ZF, em ratos, notou-se maior expressão da P450c11β-hidroxilase, enzima participante da síntese de cortisona. Entre essas zonas há uma região na qual não é expressa nenhuma das enzimas citadas e acredita-se que esta seja a região de proliferação celular (Gallo-Payet & Battista 2014; Mitani 2014). Na ZR foi detectada a expressão do citocromo b5 (CYB5) e da DHEA-sulfotransferase (SULTA1), fatores envolvidos na síntese da DHEA (Gallo-payet and Battista, 2014; Hui et al, 2009; Zhu and Garcia, 2013). Em humanos e chimpanzés também são encontradas as enzimas CYP17, uma enzima bifuncional, que atua como liase na síntese de cortisol e como hidroxilase na síntese de DHEA e, portanto, presente na ZF e na ZR; e HSD3B2, que atua na pregnenolona convertendo-a a progesterona, para a síntese de glicocorticoides e mineralocorticoides e também na conversão de DHEA a androstenediona (GALLO-PAYET; BATTISTA, 2014; PARKER et al., 2014; RAINEY; NAKAMURA, 2008). O resumo das atividades enzimáticas no processo de síntese dos esteroides de cada zona cortical é mostrado na **Figura 2.**

Ainda não está bem estabelecido o processo de formação e manutenção dessas zonas corticais (WOLKERSDÖRFER & BORNSTEIN, 1998), embora haja evidências de que exista apenas uma zona de proliferação próxima à cápsula ou entre a ZG e a ZF (CHANG et al., 2013;

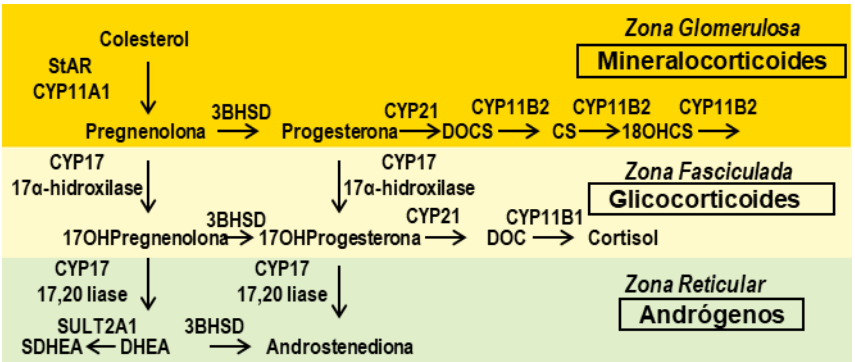
DOMENICI LOMBARDO; CORTESINI, 1988; HUANG et al., 2010; VIDAL et al., 2016) e que essas células migrem entre as zonas, segundo a teoria da "Migração celular" de Gottschau (1983) (HUANG et al., 2010). Estudos mais recentes apontam que esse processo de migração ocorre e pode estar relacionado com a formação das zonas corticais, sendo regulado pela expressão de genes *Shh (Sonic HedgeHog)* e por vias de sinalização de PKA (fosfocinase A), entre outros fatores (DUMONTET et al., 2018; HUANG et al., 2010).

Figura 1. Desenho esquemático das regiões da adrenal



Fonte: SIDIROPOULOU et al, 2018.

Figura 2. Esquema das enzimas da biossíntese dos esteroides das zonas adrenocorticais.



Fonte: Adaptado de (LEFÉVRE; BERTHERAT; RAGAZZON, 2015)

1.2 Regulação hormonal da adrenal

A atividade do córtex da adrenal é regulada pelo eixo hipotálamo-hipófise-adrenal, no qual o hipotálamo sintetiza, no núcleo paraventricular, o Hormônio Liberador de Corticotrofina (CRH), que é liberado na circulação porta-hipofisária e chega à adeno-hipófise. Na adeno-hipófise o CRH se liga ao seu receptor, que é acoplado à proteína G e, através da via de segundo mensageiro da adenilato-ciclase (AC), ativa fatores de transcrição do gene pré-pró-opiomelanocortina (POMC), que codifica o Hormônio adeno-corticotrófico (ACTH), esse tem como alvo o córtex adrenal, principalmente a ZF e a ZR. A ZG, por sua vez, é controlada, majoritariamente, pela angiotensina II (GALLO-PAYET; BATTISTA, 2014; PRESTON &WILSON, 2014). Outros fatores podem inibir ou atuar em sinergia com o ACTH como ativina, inibina e citocinas (TNF-α e leptina) (KRONENBERG et al., 2016).

O ACTH aumenta a liberação de colesterol e estimula a síntese de enzimas esteroidogênicas e de receptores de colesterol. De maneira geral, leva a um estímulo da esteroidogênese e a um crescimento no córtex adrenal, portanto, estimula tanto a síntese de glicocorticoides, quanto de andrógenos (KRONENBERG et al., 2016). Esses dois hormônios, em condições homeostáticas, são secretados em um ritmo circadiano, entretanto, existem muitas diferenças na secreção de glicocorticoides e andrógenos pela adrenal, o que indica a possível existência de um hormônio estimulador de andrógeno cortical (CASH) (KRONENBERG et al., 2016). Sabe-se também que essa glândula é sensível aos hormônios testiculares, sendo indiretamente regulada por eles (AJDŽANOVIĆ et al., 2016; BENMOULOUD et al., 2014).

1.3 Hormônios andrógenos e envelhecimento

Durante o processo de envelhecimento em machos há uma redução na secreção de testosterona nos testículos devido à redução do número das células de *Leydig* (FELDMAN et al., 2002; NEAVES et al., 1984). Sabe-se que os hormônios andrógenos influenciam o metabolismo e exercem efeitos diversos sobre diferentes órgãos e tecidos, como os do sistema musculoesquelético, o tecido adiposo e sua distribuição subcutânea, a pele e seus anexos, e as glândulas acessórias do sistema reprodutor masculino (próstata, glândula seminal, glândula coaguladora e glândula bulbouretral) além da glândula adrenal (Ajdžanović et al., 2016; Aires, 1990; Taboga, Vilamaior, Góes, 2009; Vilamaior, Taboga Carvalho, 2006). Logo, a redução desses hormônios leva a uma série de alterações fisiológicas, tanto no sistema reprodutor, como

nos demais órgãos sensíveis a esse hormônio, esse processo é denominado andropausa (CHAHAL; DRAKE, 2007).

A andropausa é uma síndrome multissintomática, que interfere nos eixos hipotalâmico-hipofisário-gonadal e hipotalâmico-hipofisário-adrenal (HATZINGER et al., 2000; MULLIGAN et al., 1997), e leva a alterações como o aumento de cortisol e redução dos andrógenos adrenais (Cusan et al., 1997; Ajdžanović et al., 2012; Heaney, Phillips, Carroll, 2012; Yiallouris et al., 2019).

Sabe-se que em humanos e símios a atividade da adrenal é alta na vida adulta e tende a decair com a senescência e são constatadas reduções na secreção dos hormônios adrenais, principalmente DHEA e SDHEA, assim como a redução da espessura da ZF e da ZR (Goncharova and Oganyan, 2017; Parker, 1999). Entretanto, em ratos há uma hipertrofia dessas zonas, bem como o aumento na concentração plasmática de corticosterona, que é o hormônio predominantemente sintetizado nessas regiões da adrenal em ratos, diferentemente dos primatas e do gerbilo, nos quais a ZF sintetiza majoritariamente cortisol e a ZR sintetiza DHEA, SDHEA e androstenediona (Rebuff et al., 1992; Cheal, 1986).

Essas diferenças na resposta ao envelhecimento no córtex adrenal entre primatas e roedores repercutem nas diferenças morfofuncionais dessa glândula entre esses animais, entretanto, sabe-se que em ratos a ZR provavelmente não sintetiza andrógenos, mas sim corticosterona, glicocorticoide mais utilizado pela espécie, diferentemente dos primatas e do gerbilo (BROCK; WATERMAN, 1999; FENSKE, 1983; OLIVER; FERNAND, 1964).

1.4 Adrenal do gerbilo

Os gerbilos da Mongólia (*Meriones unguiculatus)* possuem glândulas adrenais com características particulares, como uma alta razão entre o peso da glândula adrenal e o peso corpóreo (MALENDOWICZ, 1984), o que indica que esse é um órgão de importância fundamental para essa espécie (MALENDOWICZ, 1984). Ela possui formato triangular e alongado, coberta por uma camada de tecido adiposo. As zonas corticais são bem definidas e é visível uma zona entre ZF e a ZR com células intensamente coradas, não há evidencias da permanência de uma região similar à zona X de camundongos, no entanto não há estudos da organogênese das adrenais nessa espécie (KADIOGLU; HARRISON, 1975).

Ultraestruturalmente, o córtex adrenal também apresenta peculiaridades, como uma íntima relação entre mitocôndrias e o retículo endoplasmático, ambas organelas com

morfologia diferenciada e variável entre as zonas corticais, incluindo a presença de mitocôndrias gigantes (KADIOGLU; HARRISON, 1975). Quanto à secreção de hormônios esteroides, *M. unguiculatus* secreta majoritariamente cortisol e 19-hidroxi-11-deoxicortisol na ZF, diferentemente dos demais roedores que secretam corticosterona; enquanto na ZG e ZR sintetiza, respectivamente, mineralocorticoides e andrógenos (FENSKE, 1983; OLIVER; FERNAND, 1964).

Nosso grupo de pesquisa atua em estudos na área de desregulação endócrina no sistema reprodutor em modelos experimentais animais, principalmente em gerbilo. Muitos desses estudos utilizam animais de 12 e/ou 15 meses e investigam efeitos de diversas substâncias no desenvolvimento e no envelhecimento desses animais, pois essa espécie também é considerada um modelo adequado para estudos de envelhecimento, devido ao seu curto tempo de vida (como os demais roedores) e sua capacidade de resistência a doenças. Embora animais dessa espécie possam viver de 2 a 4 anos, aos 12 e aos 15 meses já apresentam sinais de envelhecimento em diversos órgãos, como a ocorrência espontânea de lesões na próstata (CAMPOS et al., 2008; CHEAL, 1986; VINCENT; RODRICK; SODEMAN, 1980).

Neste cenário, embora já estejam estabelecidos muitos conceitos a respeito do envelhecimento do sistema reprodutor de gerbilos, pouco se conhece sobre a atuação dos andrógenos adrenocorticais na regulação do sistema reprodutor nessa espécie, especialmente durante o processo de envelhecimento, por essa razão, é importante conhecer a morfofisiologia e o desenvolvimento do córtex adrenal, assim como seu papel na regulação do sistema reprodutor do gerbilo, o que fornecerá informações importantes sobre a complexa via de regulação endócrina desse sistema.

2 ARTIGO: "AGING EFFECTS IN ADRENAL CORTEX MORPHOPHYSIOLOGY OF MALE MONGOLIAN GERBIL'S: AN INTERESTING MODEL FOR ENDOCRINE REGULATION STUDIES"

2.1 Introduction

Since embryonic development, there is a complex endocrinological balance involving many hormones, including the steroids secreted by the adrenal cortex as mineralocorticoids, glucocorticoids, and androgens (dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and androstenedione). The adrenal androgens start to be produced at the end of the fetal period in the Fetal Zone, the primary cortical zone which originates Zona Fasciculata and Zona Reticularis (PARKER et al., 2014; PIHLAJOKI et al., 2015).

In humans at the age of three years the Fetal zone regress, and consequently the blood levels of DHEA, DHEAS (adrenal androgen most abundant in the blood), and androstenedione levels also decrease. The serum level of these hormones starts to rise again in the early adolescence, and are responsible for initiate puberty, acting in the hair growing in steroidogenic sites, behavior alterations and gonadal maturation, stimulating this to synthesize androgenic/steroidogenic hormones which culminates in the beginning of reproductive life (HUI et al., 2009).

DHEAS levels rise during adulthood and have a peak about 30 years old when it starts to decrease. Although there is a high individual variation of this hormone, previous research suggests that DHEAS can be reduced from 10 to 30% with aging, indicating a reduction of the adrenocortical activity (Parker Jr 1999; Hui *et al.* 2009; Lois *et al.* 2014). It is also known that the adrenal androgens and glucocorticoids play a key role in the reproductive system's regulation, mainly at the beginning of the reproductive age (LOIS et al., 2014; PIHLAJOKI et al., 2015).

During the aging process there is also a reduction in the testicular androgens synthesis, named as well as the other symptoms associated with this reduction as andropause or nowadays called Androgen Deficiency Syndrome, (Partial) Androgen Decline in the Aging Male (P)ADAM), Late Onset Hypogonadism (LOH), Testosterone Deficiency Syndrome (TDS) (HATZINGER et al., 2000; MULLIGAN et al., 1997; PARK; AHN; MOON, 2019; YADAV et al., 2019).

Previous research has established that there is an intimate relation between testosterone (T) levels and the adrenocortical activity, although there are few investigations of this relationship during aging process(ABOOTALEBI; KARGAR; AMINSHARIFI, 2016; LOIS et al., 2014). This lack of information happens due to, among other factors, the difficulty to find a representative experimental model, once that the morphophysiology of the adrenal cortex differs between most of the rodents and primates (PIHLAJOKI et al., 2015).

The most common experimental models of endocrine system (rats and mice) have different morphology and physiology of the adrenal cortex, since they have a lack of some steroidogenic enzymes, essential for androgens and cortisol synthesis (GALAC; WILSON, 2015; PIHLAJOKI et al., 2015). Therefore they do not secret adrenal androgens, that are produced in the Zona Reticuralis in the human adrenal cortex, and their main glucocorticoid is corticosterone instead of cortisol, major human's glucocorticoid, produced by the Zona Fasciculata (GALAC; WILSON, 2015).

Previous research has shown that Mongolian gerbil, although there is little data, has as main adrenocortical hormones cortisol and DHEA(S), and also synthesize androstenedione and aldosterone, and it has a high adrenal relative weigh (MOTZEL; WAGNER, 1992). Gerbil adrenal's morphology is also similar to primates, showing three cortical Zonas: Glomerulosa (ZG), Fasciculata (ZF) and Reticularis (ZR) (FENSKE, 1983; KADIOGLU; HARRISON, 1975; MALENDOWICZ, 1984). These rodents are used as experimental models in several studies, especially of the reproductive system in adulthood as well as in aging (CAMPOS et al., 2008; CHEAL, 1986; MALDARINE et al., 2019; PINTO-FOCHI et al., 2016; ROCHEL-MAIA et al., 2013).

Up to now, there is still little knowledge of the Mongolian gerbil's adrenal cortex physiology, especially during the aging process, therefore this study seeks to obtain data that will help to this understanding.

2.2 Material e methods

Experimental design

In this research were used thirty male Mongolian gerbils (*Meriones unguiculatus),* which were caged under constant laboratory conditions (temperature of 23ºC±2; photoperiod of 12 hours) and food and water were provided *ad libitum.* They were maintained at the bioterium of São Paulo State University (UNESP) (São José do Rio Preto) over a period of

three, six, nine, twelve and fifteen months (m), according to the experimental group (n=6). Considering that at three months old this animal are considered adults and they could live from two to four years, 12m and 15m old animals could be considered middle aged, even though some studies shows that they shows signs of senescence at these ages (CAMPOS et al., 2006, 2008; VINCENT; RODRICK; SODEMAN, 1980).

Then the animals were weighed and euthanized by anesthesia (xylazine and ketamine) overdosage followed by decapitation, their blood was collected and centrifuged at 3000 rpm for 20 min and the serum was stored at -80 °C until analysis. Their adrenal was collected, weighed and fixed in paraformaldehyde 4% for 24h. The relative weight was calculated using the body and both adrenals' weight. The procedures of this study were approved by the Ethical Committee of the São Paulo State University in São José do Rio Preto *(CEUA: 130/2016;183/2018).*

Morphological and morphometric analysis

The organs fixed were dehydrated in ethanol, clarified in xylol and embedded in Histosec (Histosec-Merck, Dermstadt, Germany). Serial sections were performed and the middle ones were chosen for morphological analysis and were rehydrated and stained with Hematoxylin and Eosin, Gomori's trichrome, and with Gömori's reticulin for collagen and reticular fibers analysis; and Gomori's Halmi trichrome for lipofuscin quantification.

The morphometrical analysis were performed in images captured by BX61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120® Virtual Microscopy Slide Scanning System (VS120-S5), using one slide per animal and 34 measurements (204 per group) of length of the three cortical zones per animal were performed in the Image Pro-Plus 6.0 software (Media Cybernetics, Inc., MD, USA)

Nuclear and cytoplasmic areas were measured in 17 images of each zone per animal captured by a camera coupled to and microscope (photomicroscope Olympus BX60, Olympus Corporation, Tokyo, Japan) under 1000x magnification, two measurements per image were performed (totalizing 204 per group) in the Image Pro-Plus 6.0 software (Media Cybernetics, Inc., MD, USA).

Lipofuscin quantification was performed in 60 images per group captured also by the slide scanning system with 400x magnification. It was used a grid with 200 points and it was counted the cells with granule which coincide with the grid, the number of points with positive cells was divided to the total in order to calculate the percentage.

Immunohistochemistry analysis

For immunohistochemistry, the rehydrated sections were submitted to the antigen retrieval with citrate or Tris-EDTA buffer in microwave (14 minutes in low power level and 7 minutes in "med-low"), then washed in phosphate buffer with tween (PBS-t) and the endogenous peroxidase block was performed in hydrogen peroxide 0,6% in methanol. A permeabilization step was performed with Triton-x 0.1% in PBS. In order to block nonspecific protein binding the sections were incubated in bovine serum albumin (BSA) 3% in PBS-t for 30 minutes, then washed in PBS-t and incubated with de primary antibody anti-AR (IgG rabbit polyclonal, N -20, sc-816, Santa Cruz Biotechnology CA, EUA) and anti-ER α (IgG rabbit polyclonal, MC-20, Santa Cruz Biotechnology, CA, EUA), diluted 1:50 in BSA1% overnight at 4ºC.

In the reaction for phosphohistone H3 (PHH3) (anti-PHH3, IgG rabbit polyclonal, #9701, *Cell signaling*, MA, EUA), cytochrome P450c17 (17-α-hydroxylase/17, 20-lyase) (anti-CYP17, IgG rabbit polyclonal, E-AB-11124, *Elabscience*) e 3β-hydroxysteroid dehydrogenase (anti-HSD3B2, IgG rabbit polyclonal, E-AB-15114, *Elabscience*) the retrieval was performed in Tris-EDTA with tween buffer at 98ºC for 1 hour, washed in PBS-t and blocked for nonspecific protein binding in BSA 3% for 1 hour, then washed in PBS-t and incubated with primary antibody (1:50) overnight at 4ºC. The block of endogenous peroxidase was performed as described above.

Both immunostainings were visualized by incubating sections with post-primarium and polymer (for 45 minutes each) and the chromogen 3,3′-diaminobenzidine-tetrahydrochloride (DAB) and counterstained with hematoxylin.

Images were captured by a camera coupled to a microscope (photomicroscope Olympus BX60, Olympus Corporation, Tokyo, Japan) under 400x magnifications. For nuclear staining it was captured 30 images of the ZR and ZF, and 42 of ZG of each group and the image-pro-plus software was also used for counting the positive nuclei for AR, ER, and PHH3, which were counted at list 2000 cells per group. For cytoplasmic immunostaining as CYP17 and HSDB3 were captured 60 images per group and the pixels intensity was measured by the ImageJ software using the plugin IHCprofiler (Varghese et al, 2014).

Apoptosis index

For the analysis of the apoptotic cells the TUNEL assay was used the ApopTag® Plus Peroxidase *In Situ* Apoptosis Detection Kit (S7101, Merck CA, USA) performed according to the manufacturer's instructions and counterstained with hematoxylin. It was captured 60 aleatory images per group, under 400x magnification and the positive cells was counted in the known area (17,59 cm^2) then was calculated the ratio of positive cells per cm^2.

Blood serum hormone assay

The serum concentrations of Testosterone, Corticosterone, and DHEAS were measured by enzyme quantitative immunoassay of capture/sandwich ELISA using specific commercial kits according to the manufacturer's instructions (Dehydroepiandrosterone Sulphate (DHEA-S) RE52181, Testosterone RE52151, and Corticosterone RE, IBL, Hamburg, Germany).

2.3 Results

Biometry

As can be seen from Table 1 (below) the body mass progressively increases until 12 months and it is smaller at 15 months. On the other hand, the adrenal absolute weight increases even more in the oldest group and the relative weight is also higher at 15m, showing continuous growth of this gland.

Morphology and morphometry

Morphological analysis shows that adrenal cortex is divided into three zones: glomerulosa, fasciculata and reticularis. The three cortical are well defined, and the ZR occupy most of the cortex, followed by ZF. In the ZR are seen two regions: one more eosinophilic and another one less eosinophilic and with a larger amount of blood vessels (**Fig. 1 and 2**).

At older ages, 12m and 15m, it was possible to observe a loss of morphological differentiation between cortical zones in some regions of the cortex; larger cells than the others in the same region, containing large amounts of lipid droplets. Regions with cell clusters with different characteristics and organization were also observed in ZF and ZR, where they express 3BHSD and CYP17 (**Fig.3**).

It is also found, at 12m and 15m, a larger amount of connective tissue between the ZR and the medulla, containing cells of ZR sparse in the middle of the connective fibers and also cells with inflammatory cell's characteristics. In this same region, near the medulla, several cells with lipofuscin granules were also observed, which were higher in the 15m old animals (**Fig.2 and 3**).

The thickness of ZG is higher at 12m and does not vary significantly in other ages, on the other hand ZF and ZR increase over the time (**Table 1**). As well as in ZR, the nuclear and cytoplasmic areas are larger in the 15m group, indicating a hypertrophy of this area throughout aging. In contrast, the ZG has a smaller nuclear area in 9m animals; and in the ZF there is no difference between the ages (**Table 1**).

Proliferation and death

The immunostaining for PHH3 has shown fairly specific to the outer ZG and mostly in the outer and middle ZR. PHH3 expression in ZR is higher in 3m, reduces at age of 6m, then increases again until 12m, and finally decreases in the older group, showing a correlation to karyometric and cytometric analysis, which ages with larger nuclear and cytoplasmic area are the same as the ones which present less immunostained cells (**Fig. 4**).

Apoptotic cells are present only in the ZR, and there are more apoptotic cells at the ages of 3m and 15m, although at 3m the proliferation is also high and at 15m there are less proliferation, showing a possible decrease in the number of cells of this area with the age.

Enzymes of androgen biosynthesis

The adrenal cortex of gerbils shows immunoreactivity for antibodies anti-human HSDB3 and CYP17, both enzymes with a main role in DHEA, androstenedione and also cortisol biosynthesis.

Expression of HSDB3 is localized most in ZF at age of 3m with some cells positives at the beginning of ZR. Interestingly with the advance of the age, most ZR shows positive cells, although it is weaker than in 3m group. Different from HSDB3, CYP17 has positive cells in the entire cortex, except for ZG and part of the medulla, whereas in older animals the beginning of ZF and the inner ZR showed stronger staining (**Fig. 5)**.

Androgen and estrogen receptor

AR expression is higher in ZG, at 3m is majority cytoplasmic, then starts to be majority nuclear with aging, being more frequent at 12m then decreases at 15m (**Fig. 6 and 8**). In the ZF there are more AR positive cells in older group, while in the ZR there are few positive cells and do not vary with aging. Similarly, the expression of ERα has no significant difference in ZF, in the ZG only 6m and 9m present difference between them; while in the ZR is higher in 3m group and decreases in the other ones, especially at 15m **(Fig**. **7 and 8**).

Blood hormones assay

Serum level of testosterone seems to be higher in 6m, 9m, and 12m, although there is no statistical difference between them. The opposite happens with DHEA-S concentrations, which is bigger at 15m **(Fig. 8)**.

2.4 Discussion

Mongolian gerbil's adrenal cortex shows morphological e functional zonation similar to humans, with the three zones (ZG, ZF and ZR), although some authors classify a fourth zone between glomerulosa and fasciculata, the zone Intermedia, which is constituted by one or two layers of flattened cells, we weren't able to classify this zone in our model, once that their morphology is not clearly established (VINSON, 2016).

The ZR in gerbils occupies the largest volume of the adrenal cortex (**Table 1**), unlike most experimental models, in which ZF occupies a larger percentage of the cortex, arising the possibility of more effective participation of this gland in the reproductive system endocrine control through the production of androgens. This possibility can also be evidenced by the hypertrophy of this zone and of the adrenal cortex observed with aging.

Prior studies that have noted the importance of adrenal cortex role in the regulation of testicular androgen and estrogens, as well as in the production of androgens, glucocorticoids and mineralocorticoids, which present a cross-talk reported in other studies, in different species, including rodents relatively phylogenetically close to the gerbils (AJDŽANOVIĆ et al., 2016; BENMOULOUD et al., 2014; RAYNAUD; MÜLLER; SCHRADIN, 2012; ZATRA et al., 2018).

The increase in the absolute and relative weight of this gland, as well as the larger thickness of the cortical zones, lipid accumulation in the ZF and ZR cells and the presence of proliferative cells up to 15m, are indicative that this gland maintains its function throughout the

aging, and presents hypertrophy possibly as a compensatory mechanism for low cellular metabolism, whereas DHEA (S) levels are reported to be decreasing in aging in humans (HEANEY; PHILLIPS; CARROLL, 2012; LABRIE et al., 1997).

This study describes, for the first time, cell proliferation in the gerbil adrenal cortex using immunohistochemistry for PHH3, a proliferation-specific marker that identifies chromosomal condensation, thus only after the G2 phase of the cell cycle, also used as a marker for carcinoma in various organs (HENDZEL et al., 1997; KIM et al., 2017; NOWAK et al., 2014). Two regions of proliferation were observed: one between the connective tissue capsule and the ZG and another in the ZR, similar to that described in mice (CHANG et al., 2013). As well as apoptotic cells were only observed in this zone, corroborating to the migration theory (HUANG et al., 2010).

In the ZR a region with a high percentage of proliferative cells was found, which differs from what has been reported in other rodents, such as mice, where there are few proliferative cells in this region and in rats, where they are only found in animals subjected to stress (CHANG et al., 2013; ZAKI et al., 2018). However, these studies used another marker for proliferation, Ki67, also specific and widely used. Thus, it is possible that this proliferation region does not exist in rats and mice since studies in adrenocortical carcinoma show that Ki67 and PHH3 markings have a high correlation (DUREGON et al., 2014).

The hypertrophy found in the adrenal cortex throughout aging is very similar to that found in response to the depletion of testicular androgens (orchiectomy) already reported in rats and desert rodents, showing that hypertrophy and maintenance of growth of this gland in aging correlates with the reduction of testicular androgens (AJDŽANOVIĆ et al., 2016; BENMOULOUD et al., 2014). In this context, another aspect that corroborates with the literature is that the expression and alterations of expression of androgen and estrogen receptors founded in aging are similar to those described in orchiectomy (AJDŽANOVIĆ et al., 2016; BENMOULOUD et al., 2014).

Data from the expression of AR in *M. unguiculatus* were similar to those found in another species of desert rodent, *Psamommys obesus*, with more marked cells in ZG and few in ZF and ZR (BENMOULOUD et al., 2014); differing from what was found in rats, which AR expression is similar in the three cortical zones. Likewise, the AR expression increased in the ZG and ZF in the older animals (12 and 15 months old), although it maintained unaltered in the ZR, even though the T levels do not have decreased. Considering that T plays a role in the regulation of the adrenal cortex, these results may indicate that adrenal cortex is sensitive to

slight variations of this hormone.(AJDŽANOVIĆ et al., 2016). According to Benmouloud (2014), after orchiectomy the expression of this receptor rises, evidencing an upregulation of AR from testosterone (BENMOULOUD et al., 2014).

Another steroid hormone receptor found in the adrenal, the estrogen receptor, is poorly studied, and there are no published data on this receptor in desert rodents and little data in other experimental models. In humans and rats, the presence of genes and mRNA for both estrogen receptors (ESR1 and ESR2), encoding ERα and ERβ, respectively, was found in the adrenal gland (KUIPER et al., 1997; TREJTER et al., 2015). In rats was detected by immunohistochemistry the presence of ERα only after orchiectomy and testosterone treatment (AJDŽANOVIĆ et al., 2016). On the other hand, in the present study, ERα expression was found in all regions of the adrenal cortex, such divergence may be correlated with the presence of the 3BHSD enzyme in gerbils. This enzyme is known to a role in one of the mechanisms by which dihydrotestosterone (DHT) acts on the adrenal via ER since this enzyme is capable of converting DHT to 3β-diol (LUND et al., 2004).

In this context, it is known that the enzyme 3BHSD, as well as CYP17, participate in the synthesis of both adrenal androgens produced in ZR and cortisol, which may shed light on the relationship between these hormones, since cortisol may inhibit the synthesis of androgens and vice versa (Ajdznovic et al., 2015; Benmouloud et al., 2014; Raynaud et al., 2012; Zatra et al, 2018). Otherwise, experimental models such as the Wistar rat and mice do not have these enzymes, indicating that there is no synthesis of adrenal androgens, or that it occurs by another pathway (PIHLAJOKI et al., 2015; VINSON, 2016).

In gerbil, the expression of these enzymes occurred in regions similar to those found in humans, 3BHSD mostly in ZF and CYP17 equally expressed in ZF and ZR (NAKAMURA et al., 2015; REGE et al., 2014). Again, the synthetic pathways of gerbil differ from rats and mice, whose expression of these enzymes has not been found; demonstrating that Mongolian gerbils potentially produce DHEA, androstenedione, and cortisol (PIHLAJOKI et al., 2015; VINSON, 2016). These findings highlight the Mongolian gerbil adrenal as a valuable model for the study of endocrine regulation and endocrine disruption.

In this scenario, the model analyzed here also proves to be an interesting model in the study of aging, since the labeling for HSDB3 also varied with age, and at 3 months it is localized, mostly in the ZF and in some ZR cells, however with advancing age the whole ZR present positive cells, although apparently weaker than 3m. These changes in the expression of this enzyme are reported as part of the adrenarche process, which consists of the onset of adrenal

secretion of androgens, which leads in humans and chimpanzees to the first signs of puberty and the subsequent rise in testosterone levels (BAQUEDANO; BELGOROSKY, 2018; RAINEY; NAKAMURA, 2008)

Another similarity in gerbil adrenal with primates is the presence of detectable serum levels of the most abundant circulating androgen produced by adrenal, DHEAS and also higher cortisol production in detriment of corticosterone (FENSKE, 1983). The expression of these enzymes shows that it is regulated by serum testosterone levels, especially HSDB3, for which there are reports of inhibition by T, corroborating the apparent reduction of enzyme expression in 6 months old animals, when also serum T levels are high (Nowak et al., 1995; Stalvey, 2002; Zatra et al., 2018).

T and DHEA(S) have a correlated action, since gonadal steroids act in the hypothalamus-pituitary-adrenal axis, regulating adrenocortical activity (HANDA; WEISER, 2014). According to previous studies, increased concentrations of both hormones at puberty and a decline in aging may occur concomitantly (FELDMAN et al., 2002; HEANEY; PHILLIPS; CARROLL, 2012; LABRIE et al., 1997). This correlation can be observed in gerbils through the expression of DHEA biosynthesis enzymes, which at 3m has a higher expression of HSDB3 than at 6m, while T is higher at 6m and thereafter keep it high up to 12m, later decreases slightly, which is accompanied by an increase in DHEAS. These results, as well as the reduction in cell proliferation and androgen receptor expression at the ages that T is highest, show the fine regulation of adrenal cortex physiology by T.

This morphophysiological alterations show that the adrenal cortex is sensitive to T variations, and may indicates an initiation of an andropause-like process in this specie, although it does not appear significantly in the serum levels analysis probably in order to the small number of animals and the individual variation of this hormone. This process consists of the reduction of testicular androgens that occurs with aging and the consequent reduction of hormone production by the testes (ABOOTALEBI; KARGAR; AMINSHARIFI, 2016). Although the gerbil life curve is not well established, it is known that it lives around two years and at 12 months old it already shows signs of senescence in various organs of the reproductive system, especially the prostate (CAMPOS et al., 2006, 2008; CORDEIRO et al., 2008; CORRADI et al., 2009; PEREZ et al., 2016; SCARANO; VILAMAIOR; TABOGA, 2006). The signs of senescence were also noted in the morphology of the adrenal gland, since there was an increase in collagen fibers, regions with different tissue organization and, especially,

lipofuscin accumulation, which is evident from 12m and becomes even more prominent at 15m and the increase of cell death at the age of 15m.

2.5 Conclusion

This study reported the similarities of the Mongolian gerbil adrenal cortex with primates, through morphology, expression of androgen and cortisol biosynthesis enzymes, androgen and estrogen receptors. Thus, described the morphophysiology of this gland from adulthood to aging also showed similarity, although further studies are still needed to establish whether the hormonal variations found in this model can, in fact, be characterized as andropause, adrenarche, and adrenopause.

This study has identified that adrenal from male gerbils 12m and 15m show characteristic signs of senescence, such as lipofuscin accumulation and hypertrophy, which is possibly a compensatory response to decreased metabolism and circulating androgens.

The present study provides the first comprehensive assessment of the complex morphophysiology of the Mongolian gerbil adrenal cortex throughout aging, providing information indicating that this species is a possible experimental model for studies of the adrenal gland and aging.

Table 1 – Biometry

Age (months)		3	6	9	12	15
Body mass(g)		71,43 ±4,99[a]	91,43 ±11,12 [b]	108,9 ±6,82 [b,c]	113,3 ±10,69 [c]	100,1 ±3,38 [b,c]
Absolute adrenal gland weight (g)		0,023 ±0,003[a]	0,038 ±0,005[b]	0,041 ±0,006[b]	0,040 ±0,009[b]	0,055 ±0,007 [c]
Relative Adrenal gland weight (% w:w)		0,036 ±0,007[a]	0,041 ±0,006 [a]	0,038 ±0,007 [a]	0,035 ±0,006 [a]	0,055 ±0,0008[b]
	capsule	11,810 ±5,376[a]	9,391 ±3,548 [b]	8,128 ±2,972[c]	8,436 ±2,728[b,c]	8,948 ±3,344[b,c]
Cortical regions length (µm)	ZG	37,69 ±10,37[a]	35,60 ±8,200[a,c]	34,84 ±8,559[a]	41,12 ±9,712[b]	38,49±10,240[c]
	ZF	172,60 ±67,34[a]	220,40 ±56,86[b]	223,6 ±72,22[b]	233,9 ±62,82[c]	218,4 ±59,26[b]
	ZR	405,30 ±154,40[a]	570,20 ±155,80[b]	613,2 ±137,40[c]	660,8 ±164,80[c,d]	700,8 ±192,70[d]
Nuclear área (µm²)	ZG	24,23 ±4,858[a]	23,03 ±4,664[a,c]	19,47 ±4,316[b]	21,98 ±4,683[c]	27,06 ±5,956[d]
	ZF	28,21 ±5,229[a]	25,01 ±3,566[b]	24,74 ±4,558[b]	26,72 ±5,150[c]	28,27 ±5,780[a]
	ZR	26,57 ±6,544[a,b]	27,66 ±5,479[a,b]	25,52 ±4,891[b]	27,13 ±5,519[a]	31,74 ±6,275[c]
Cytoplasmic área (µm²)	ZG	67,06 ±15,66[a]	74,58 ±17,53[b]	64,25 ±15,69[a]	73,51 ±20,48[b]	86,86 ±19,64[c]
	ZF	147,1 ±37,29[a]	185,3 ±53,10[b]	178,0 ±48,91[b]	197,3 ±48,53[c]	189,0 ±42,06[c,d]
	ZR	96,79 ±26,73[a]	118,5 ±30,68[b]	109,6 ±28,91[c]	111,7 ±24,96[b,c]	118,5 ±29,99[b]
Nuclear/ cytoplasmic ratio	ZG	0,371 ±0,074 [a]	0,316 ±0,064 [b]	0,310 ±0,073 [b]	0,306 ±0,071 [b]	0,318 ±0,068 [b]
	ZF	0,2038 ±0,053[a]	0,1454 ±0,033[b,c]	0,1457 ±0,038[b,c]	0,1411 ±0,036[b]	0,1538 ±0,037[c]
	ZR	0,2813 ±0,056[a]	0,2473 ±0,055[b]	0,2386 ±0,041[b]	0,2492 ±0,043[b]	0,2770 ±0,053[a]

Data expressed in mean ± SD, submitted to ANOVA's test followed by Tukey's, considering p≤0,01. a ≠b≠c≠d**.** Font: Author.

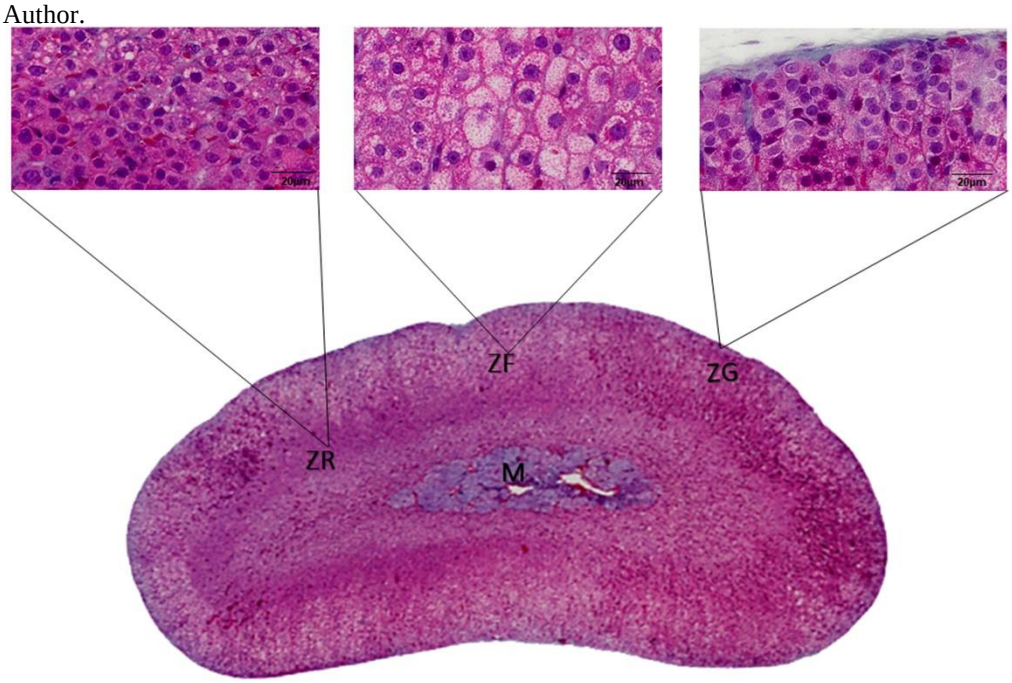


Figure 1 – Photomicrography of the longitudinal section of the adult gerbil adrenal gland (3m old), stained by the technique of Tricômico de Gömori. ZR: Zona Reticular; ZF: Zona Fasciculada; ZG: Zona Glomerulosa; M: Medulla. Font: Author.

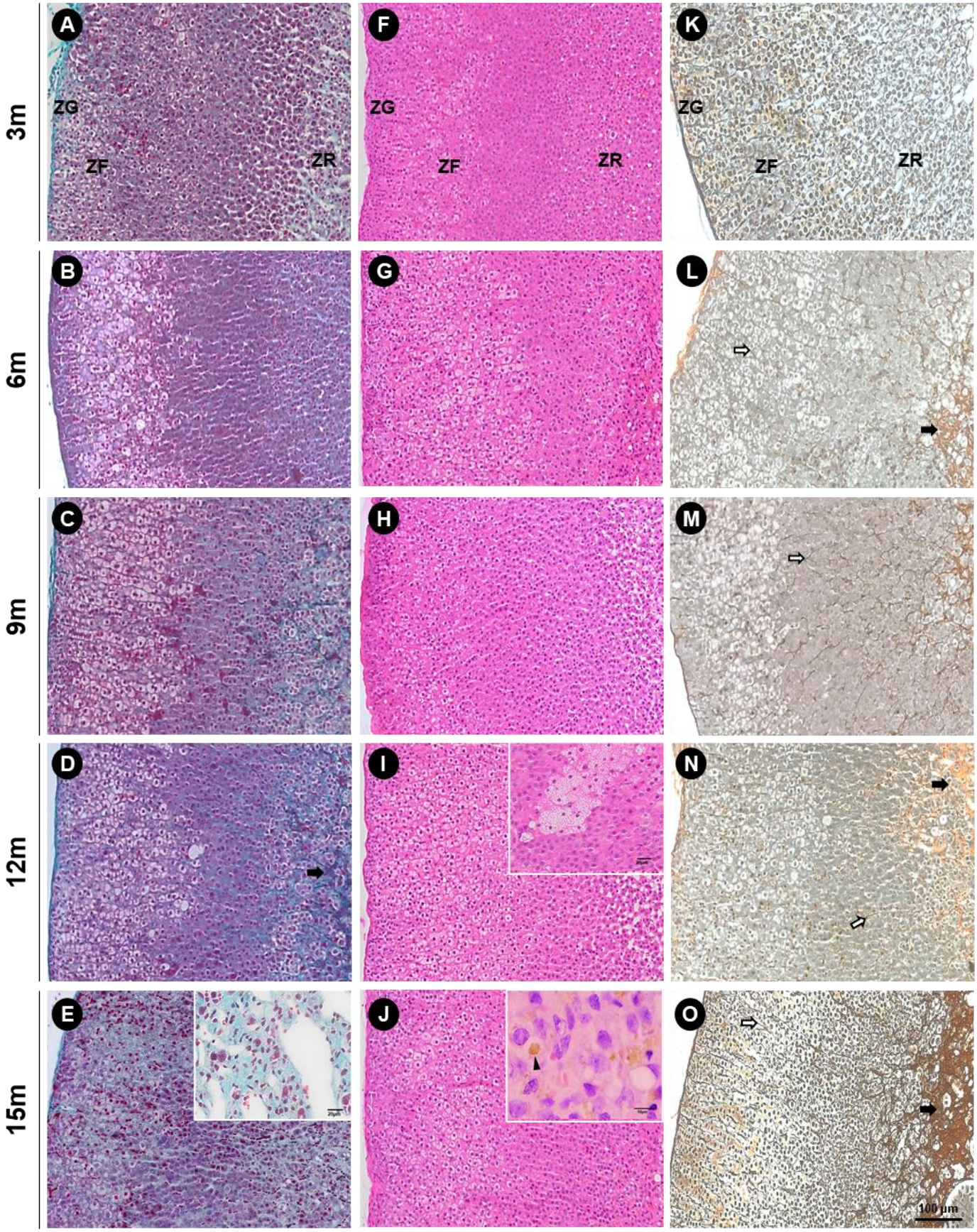


Figure 2 - Adrenal cortex general morphology in aging. Photomicrography stained with Gömori's Trichrome in the first column, H&E in the second and Gömori's Reticulin in the third. In the Gömori's reticulin is possible to see reticular fibers (white arrows) and collagen II fibers (black arrows), which there are more at 15m old group. Insets: E. Large connective tissue between the ZR and medulla; I. hypertrophic cells with large amounts of lipid droplets; J. Section showing lipofuscin granules (triangle) in the ZR's cells. Font: Author.

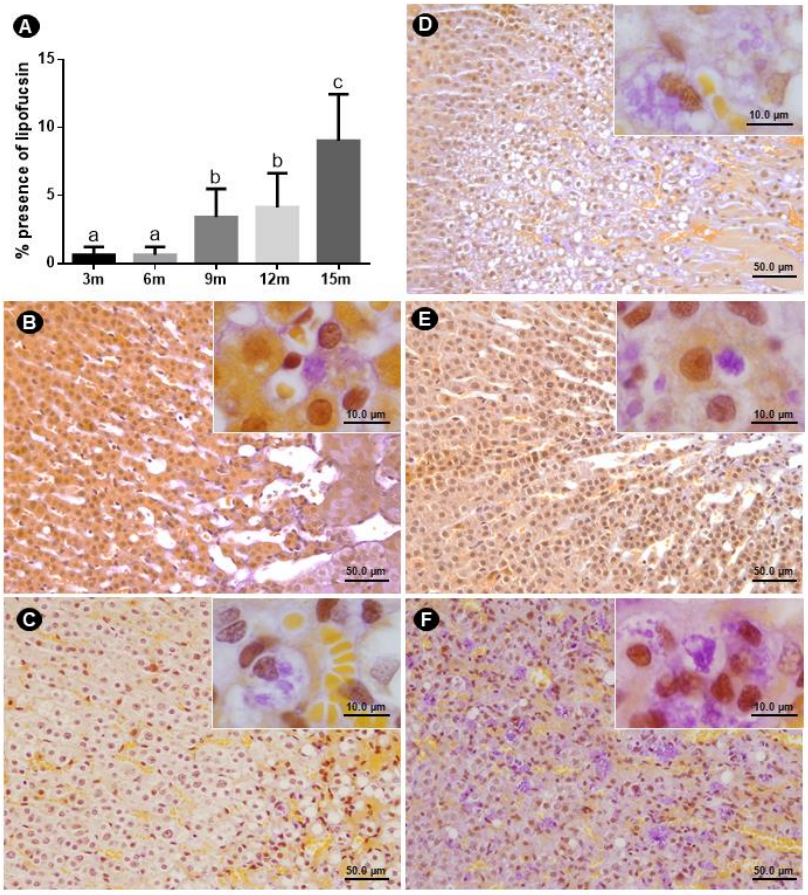


Figure 3. a. Percentage of lipofuscin, data expressed in mean±SEM, a≠b≠c, considering p≤ 0,05, submitted to Kruskal-Wallis test with post hoc of Dunn. **b.** 3m, **c.** 6m; **d.** 9m; **e.** 12m; **f.** 15m; all images have insets of the granuli of lipofuscin in the ZR. Font: Author.

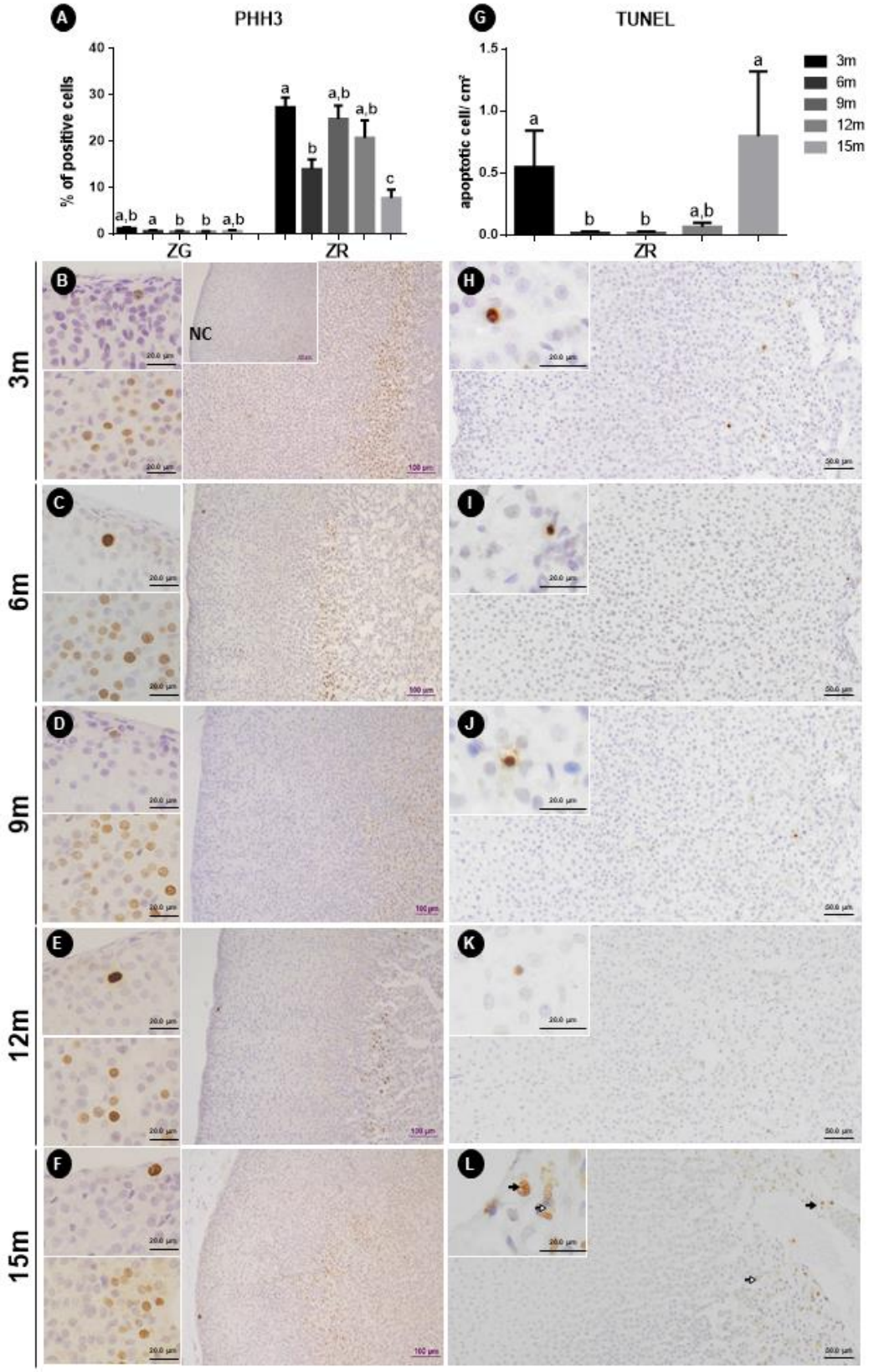


Figure 4 – a. Percentage of PHH3-positive cells, data expressed in mean ± SEM, a≠b≠c, considering p≤ 0,05, submitted to Kruskal-Wallis test with post hoc of Dunn. **B-F.** Photomicrographs of PHH3 immunohistochemistry in the different ages, all images of the first column have insets of the ZG in the top left corner and of the ZR in the bottom; **G.** Ratio of apoptotic cells per cm², data expressed in mean ± SEM, a≠b considering p≤ 0,05, data submitted to Kruskal-Wallis test with post hoc of Dunn. **H-L.** Photomicrographs of TUNEL reaction in the different ages, insets of positive cells in the ZR. **NC:** Negative Control; **Black arrow**: Apoptotic cell; **White arrow**: Lipofuscin. Font: Author.

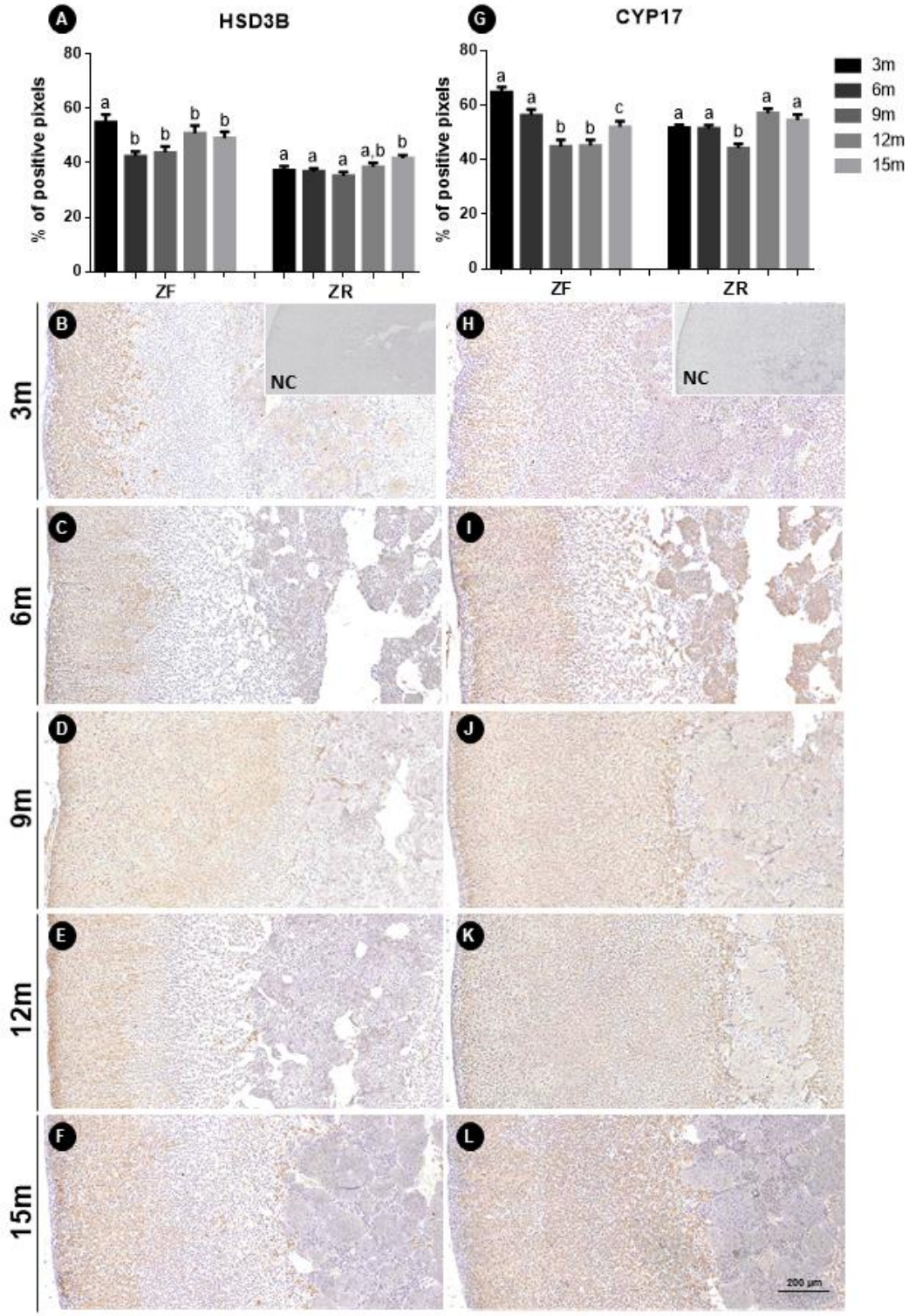


Figure 5 – A. Percentage of positive pixels to the imunnohistochemistry for HSD3B2 expressed in mean ±SEM, a≠b≠c considering p≤ 0,05, data submitted to Kruskal-Wallis test with post hoc of Dunn. **B-F.** Immunoreaction for HSD3B through the adrenal in the different ages. **G.** Percentage of positive pixels to the imunnohistochemistry for CYP17 expressed in mean ±SEM, a≠b≠c considering p≤ 0,05, data submitted to Kruskal-Wallis test with post hoc of Dunn. **H-L.** Immunoreaction for CYP17 through the adrenal cortex in the different ages. **NC:** Negative Control. Font: Author.

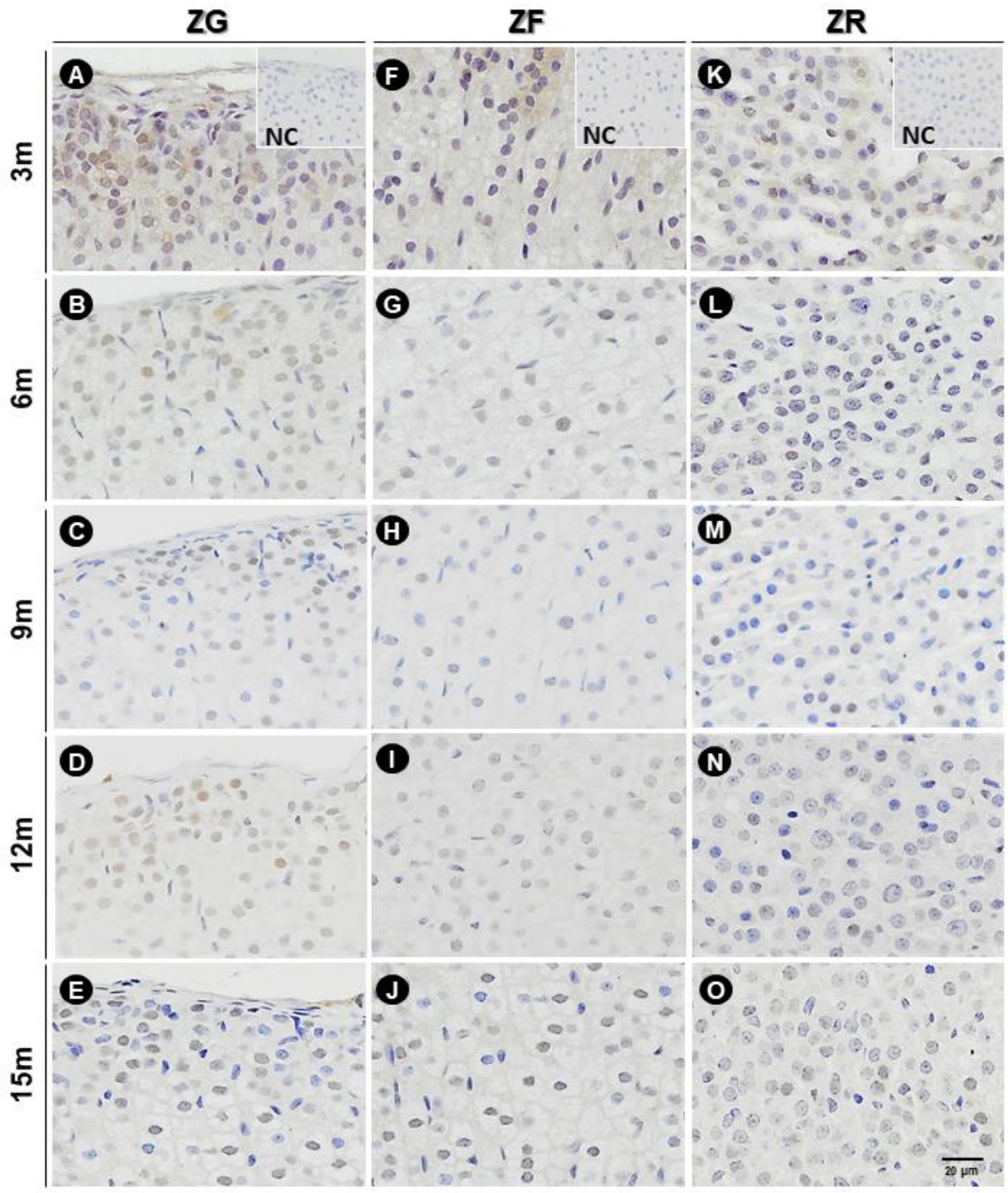


Figure 6 –AR immunostained cells in the three cortical zones. NC: Negative Control. Font: Author.

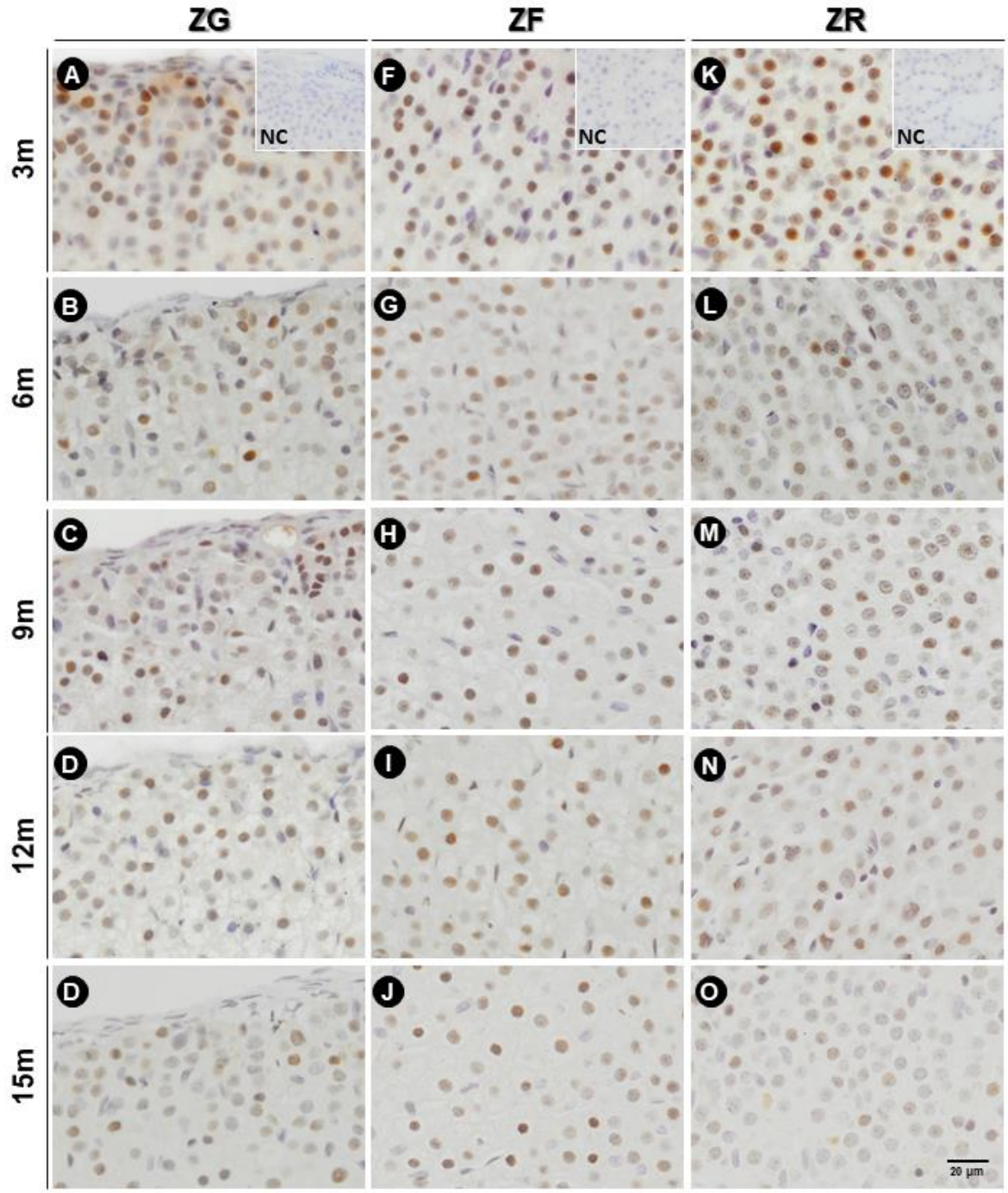


Figure 7 –ERα immunostained cells in the three cortical zones. NC: Negative Control. Font: Author.

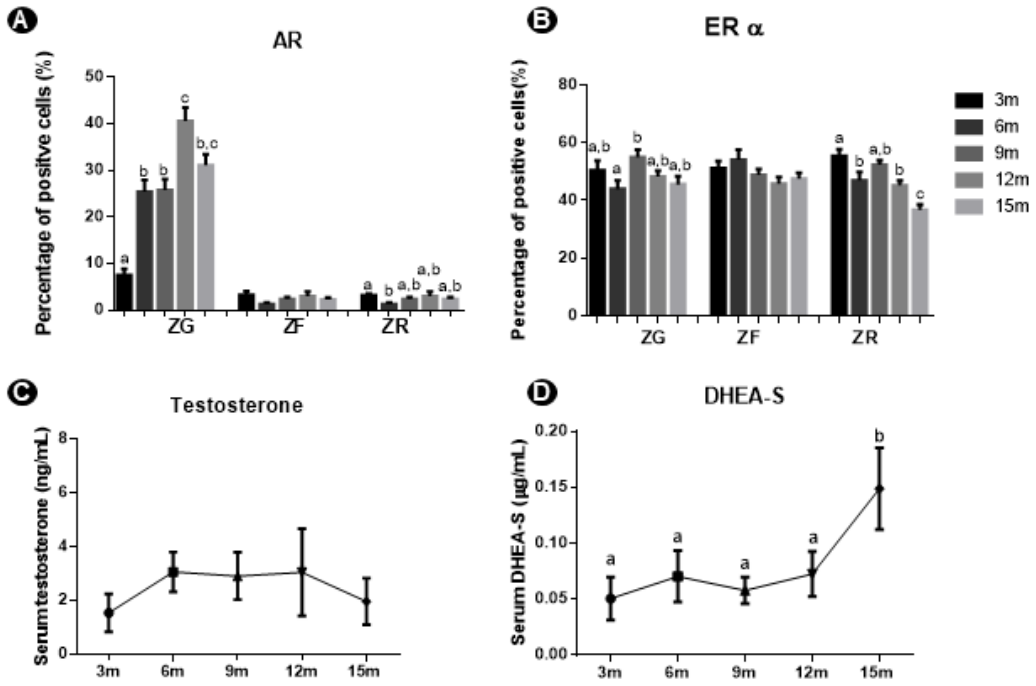


Figure 8 – A, B. Percentage of AR and ERα positive cells, data expressed in mean ± SEM, a≠b≠c, considering p≤ 0,05, submitted to Kruskal-Wallis test with post hoc of Dunn. **C, D.** Serum testosterone and DHEA-S levels. Data expressed in mean ± SD, submitted to ANOVA's test followed by Tukey's, a ≠b considering p≤0,05**.** Font: Author.

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3 CONCLUSÃO

Esse estudo relatou as similaridades do córtex adrenal de gerbilo da Mongólia com primatas, desde a morfologia, expressão de enzimas da biossíntese de andrógenos e cortisol e receptores de andrógenos e estrógenos. Assim descrita a morfofisiologia dessa glândula desde a idade adulta até o envelhecimento também mostrou semelhança, embora estudos complementares ainda sejam necessários para estabelecer se as variações hormonais encontradas nesse modelo podem ser, de fato, caracterizadas como andropausa, adrenarca e adrenopausa.

Foi constatado que a adrenal dos gerbilos machos de 12m e 15m apresentam sinais característicos de senescência, como o acúmulo de lipofuscina e hipertrofia, que é, possivelmente, uma resposta compensatória ao decréscimo do metabolismo e dos andrógenos circulantes.

Os resultados dessa pesquisa norteiam uma compreensão inicial da complexa morfofisiologia do córtex adrenal do gerbilo da Mongólia durante o envelhecimento, fornecendo informações que indicam que essa espécie seja um possível modelo experimental para estudos da glândula adrenal e de envelhecimento.

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