**Original article**

**DOI: 10.5633/amm.2024.0110**

# CORRELATION ANALYSIS OF PITUITARY LUTEINIZING AND SOMATOTROPIC CELLS IN MEN DURING AGING

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**CORRELATION ANALYSIS OF PITUITARY LUTEINIZING AND SOMATOTROPIC CELLS IN MEN DURING AGING**

This manuscript focused on examining the correlation of immunoreactive luteinizing (LH) and immunoreactive somatotropic (GH) cells in men during aging. Anti-LH and anti-GH are the antibodies used to label the mentioned pituitary cells in 14 male cadavers. The cells identified in this way were analyzed with ImageJ. The obtained results were statistically analyzed using the SPSS statistical software package. The results of the morphometric analysis showed that during aging, the surface area of LH and GH cells increased significantly ($p<0.05$), and that the nuclear-cytoplasmic ratio decreased, and that the obtained changes were particularly significant ($p<0.05$) in elderly cadavers over 70 years of age. These results showed that after the mentioned period, there was a hypertrophy of the examined cells. The resulting changes were of a functional nature and showed that cadavers after the age of 70 have a significantly reduced hormonal capacity. Based on this, it can be concluded that the investigated morphometric parameters of gonadotropic LH and GH cells correlate significantly, which indicates the parallel occurrence of adaptation and compensatory mechanisms in these cells in men during aging.

***Key words****: aging; men; LH cells; GH cells; immunohistomorphometry*

**Originalni rad**

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# *KORELACIONA ANALIZA LUTEINIZIRAJUĆIH I SOMATOTROPNIH ĆELIJA HIPOFIZE KOD MUŠKARACA TOKOM STARENJA*

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***KORELACIONA ANALIZA LUTEINIZIRAJUĆIH I SOMATOTROPNIH ĆELIJA HIPOFIZE KOD MUŠKARACA TOKOM STARENJA***

Ovaj rukopis se fokusirao na ispitivanje korelacije imunoreaktivnih luteinizirajućih (LH) i imunoreaktivnih somatotropnih (GH) ćelija, kod muškaraca tokom starenja. Anti-LH i anti-GH su antitela koja su obeležila navedene ćelije hipofize kod 14 muških leševa. Ćelije identifikovane na ovaj način analizirane su sistemom ImageJ. Dobijeni rezultati su statistički analizirani pomoću statističkog softverskog paketa SPSS. Rezultati morfometrijske analize su pokazali da se tokom starenja površina LH i GH ćelija značajno povećala ($p<0.05$), a da je nuklearno-citoplazmatski odnos smanjen i da su dobijene promene posebno značajne ($p<0.05$) kod leševa starijih od 70 godina. Ovi rezultati su pokazali da je nakon navedenog perioda, došlo do hipertrofije ispitivanih ćelija. Nastale promene su bile funkcionalne prirode i pokazale da leševi posle 70. godine imaju značajno smanjen hormonski kapacitet. Na osnovu ovoga se može zaključiti da ispitivani morfometrijski parametri gonadotropnih LH i GH ćelija značajno koreliraju što bi ukazivalo na paralelnu pojavu adaptacionih i kompenzacionih mehanizama u ovim ćelijama kod muškaraca tokom starenja.

***Ključne reči:*** *starenje, muškarci, LH ćelije, GH ćelije, imunohistomorfometrija*

**Introduction**

Aging in humans leads to physical, mental and functional changes over time which is reflected in the dysfunction of the neuroendocrine system (1). The aging process has always attracted the attention of many researchers, especially in recent decades when people's life expectancy has increased (2). Therefore, the study of endocrine regulation of the aging process occupies a significant place in many scientific studies of researchers around the world (3). Late hypogonadism or gonadopause is characterized by progressive dysfunction of the hypothalamus-pituitary-testis system, reduction of maximum and average luteizing hormone (LH) pulse amplitude, reduction of LH concentration and reduction of negative feedback mediated by testosterone (4). Literature data show that aging is not associated with any measurable disturbance of gonadotropic secretion either active or immunologically reactive LH cells (5). The causes of such progressively dysregulated LH secretion and testosterone release are still unclear. Decreased expression of androgen receptors in the brain and pituitary gland in older men may be the reason for impaired testosterone feedback efficiency (6).

The activity of the somatotropic axis changes during life. Hull and Harvey (7) have suggested that growth hormone (GH) may affect gonadal function by increasing gonadotropin secretion in the hypothalamus and pituitary gonadotropic cells. GH secretion rises during gestation, falls during the neonatal period, remains stable during childhood, rises during puberty, and afterwards drops during the adult life (8). It has been observed that the synthesis of GH decreases by 14% per decade, and that over 35% of men have a deficiency of this hormone at the age of 60 (9). GH levels decline significantly in people over 70 years of age, and represent about one third of the value compared to late puberty (10). The causes and mechanisms responsible for somatopause and late hyposomatotropism have not been sufficiently studied and described in the literature.

Based on these and other observations in the literature, it is clear that functional disorders during aging at the level of two human anabolic axes, gonadotropic and somatotropic, are currently not adequately histomorphologically analyzed and described. Precisely because of the many unknown facts about the relationship between LH and GH cells during aging, the focus of this work was to examine the correlation of immunohistological characteristics of gonadotropic LH and GH cells in men during aging.

**Materials and methods**

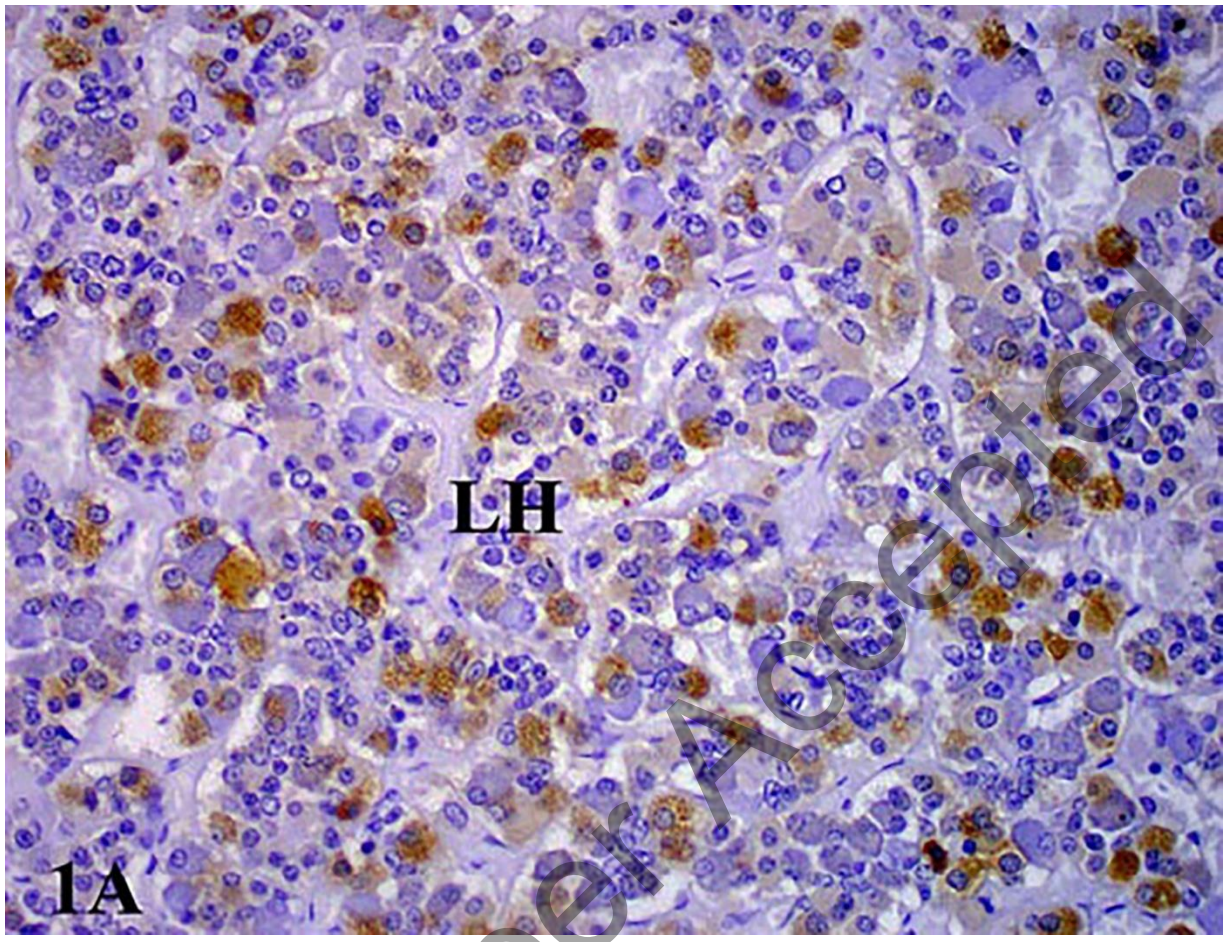
Pituitaries for this research were taken from 14 male cadavers aged 41 to 87 years. The cadaveric material was taken in accordance with the ethical norms approved by the Ethics Committee of the Faculty of Medicine of the University of Niš (Decision No. 12-2307-2/8 of 10 March 2016), which regulates the use of cadaveric material in biomedical research. Autopsy material was taken during a routine autopsy at the Center for Forensic Medicine in Nis, Serbia, within a period of no longer than 24 hours. The cadavers used in this study had not been diagnosed with neurological, endocrine or psychiatric disorders during life. No visible damage to the brain or pituitary gland was observed during the autopsy. The obtained cadaveric material was divided into groups that were described in detail in our earlier work (11). Isolated pituitary glands of male cadavers were immunohistomorphometrically processed according to a previously established procedure (12-15).

*Statistical analysis*

All obtained results were statistically processed using the SPSS software package (version 16) and analyzed using One Way ANOVA and Tukey-Kramer post hoc test.

**Results**

In the pars distalis of the pituitary gland of men aged 47 and 87 years, LH cells were oval or polygonal in shape and centrally scattered in the lateral wings of the adenohypophysis, while in the mucoid, wedge-shaped part of the gland these cells were localized within acinar formations (Fig. 1A, B). The cytoplasm contained numerous secretory granules and was darkly stained, with a markedly granular appearance (Fig. 1A, B). In 87-year-old men, more numerous, oval LH cells had eccentrically smaller, hyperchromatic, immunonegative nuclei compared with younger cases (Figure 1B). Immunoreactive GH cells were polygonal in shape and with an eccentric euchromatic nucleus (Fig. 2A, B). In younger cases, GH cells were rare and scattered in the pars intermedia, while their presence in the lateral wings of the adenohypophysis was much more noticeable (Figure 2A). In older cases, these cells were larger, showing a slightly stronger immunoreactive reaction in the pars intermedia of the adenohypophysis (Figure 2B).



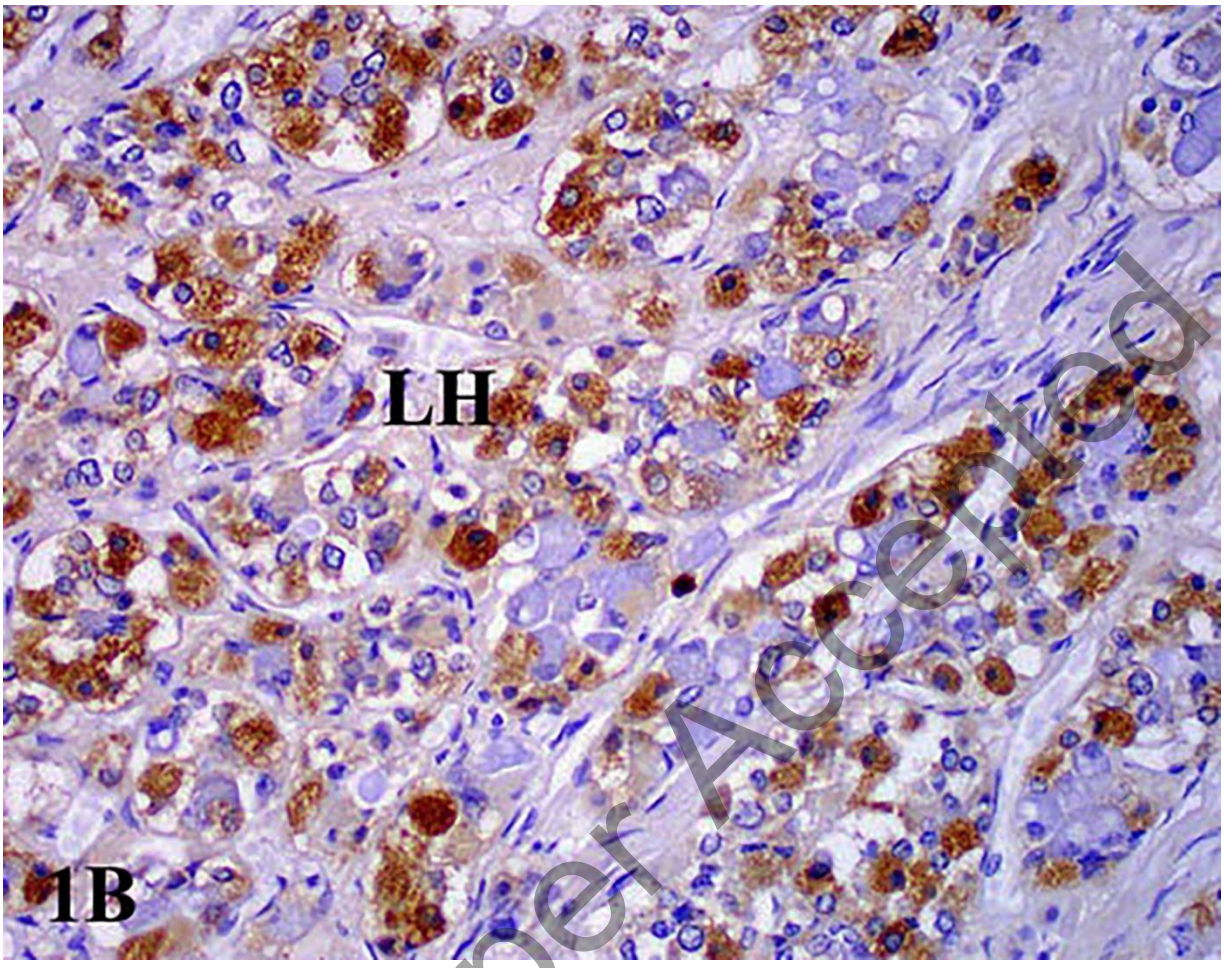
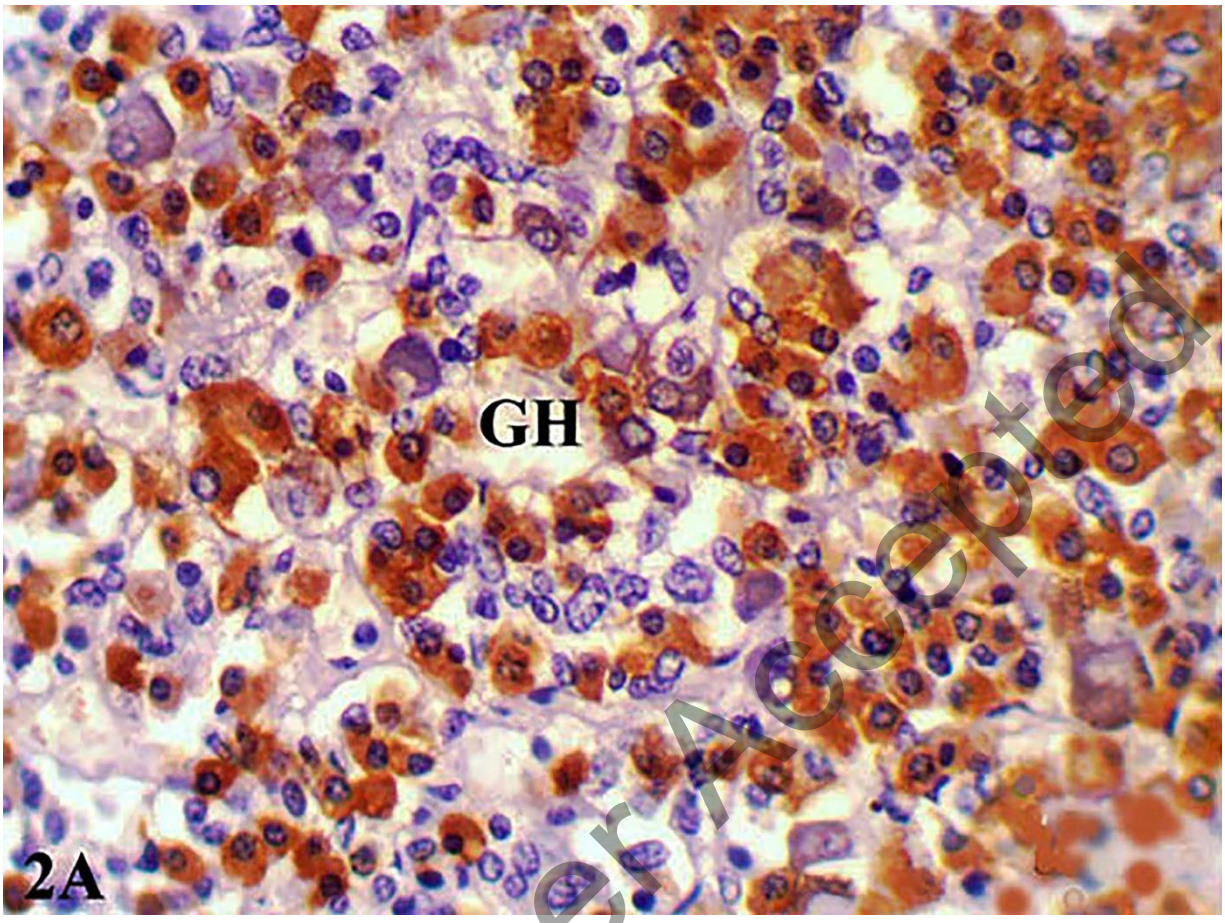



**Figure 1.** Representative micrographs of immunopositive LH cells in a 41 years old man, with eccentric or central euchromatic nuclei (marked with arrows) (A); large immunopositive LH cells with small eccentric hyperchromatic nuclei in a 87 years old man (B), PAP, objective lens magnification 10x.



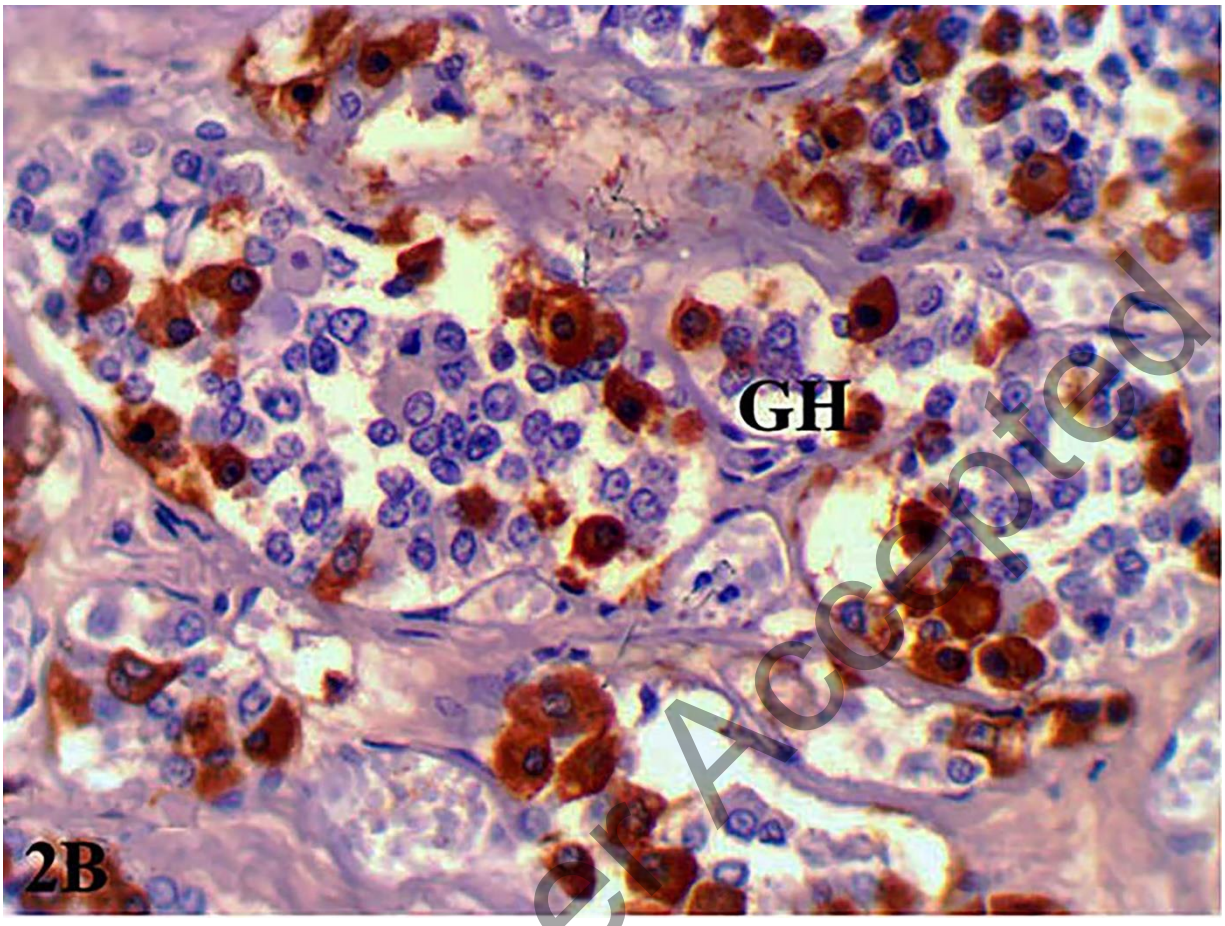



**Figure 2.** Representative micrographs of immunopositive GH cells in younger man: these are predominantly polygonal, with eccentric euchromatic nuclei (A); large GH cells with immunopositive cytoplasm, with eccentric hyperchromatic immunonegative nuclei in an older man (B). PAP 40x.

Table 1. shows the correlation of morphometric parameters of LH and GH pituitary immunoreactive cells in the analyzed cases. The area and nuclear-cytoplasmic ratio of LH cells statistically significantly ($p<0.05$) correlated with the same parameters of GH cells. Other morphometric parameters did not show a statistically significant correlation between these two groups of cells.

**Table 1**. Correlation between morphometric parameters of gonadotropic LH and somatotropic cells of the adenohypophysis in the analyzed cases

| Parameter | | $A_{GH}$ | $A_{NGH}$ | $(N/C)_{GH}$ | $V_{VGH}$ |
|---|---|---|---|---|---|
| | R | 0.69 | -0.1 | -0.65 | -0.02 |
| $A_{LH}$ | p | 0.006 | 0.74 | 0.012 | 0.94 |
| | N | 14 | 14 | 14 | 14 |
| | R | 0.36 | 0.34 | -0.01 | -0.24 |
| $A_{NLH}$ | p | 0.2 | 0.24 | 0.96 | 0.41 |
| | N | 14 | 14 | 14 | 14 |
| | R | -0.47 | 0.31 | 0.65 | -0.14 |
| $(N/C)_{LH}$ | p | 0.09 | 0.29 | 0.012 | 0.64 |
| | N | 14 | 14 | 14 | 14 |
| | R | 0.38 | 0.06 | -0.31 | 0.21 |
| $V_{VLH}$ | p | 0.18 | 0.83 | 0.28 | 0.47 |
| | N | 14 | 14 | 14 | 14 |

$A_{GH}$– area of GH cells; $A_{NGH}$ – area of GH nuclei; (N/C)GH – nuclear–cytoplasmic ratio of GH cells; $V_{VGH}$ – volume density of GH cells; $A_{LH}$ – area of LH cells; $A_{NLH}$ – area of LH nuclei; (N/C)LH – nuclear–cytoplasmic ratio of LH cells; $V_{VLH}$ – volume density of LH cells



**Discussion**

During aging, somatopause occurs, which is characterized by a decrease in the level of GH in the blood (16). The correlation between reproductive status and GH secretion, indicates that GH may have a modulating role during this process (7). Osamura and Watanabe (17) showed that in the normal adult anterior pituitary, about 10% of GH cells contained beta LH and alpha and beta follicle-stimulating hormone (FSH) subunits, and had an appearance that suggested coexistence of GH with gonadotropic LH and FSH.

Changes in the adenohypophysis are a link in the chain of changes that include the hypothalamic-pituitary axis, hypothalamic-somatotropic axis and hypothalamic-pituitary-adrenal axis (16, 18-19).

The analysis of immunohistochemically labeled LH and GH cells of the pituitary gland of an 87-year-old man showed that they were larger, more often oval and with an eccentric, smaller, hyperchromatic nucleus compared to younger cases. The obtained results of the analysis of gonadotropic LH cells were in accordance with our previous work (14). In younger cases, GH cells showed a slightly stronger immunopositive response in the pars intermedia of the adenohypophysis. Results similar to ours were obtained by Antić et al. (20), who examined immunoreactive GH cells in 27 cadavers of both sexes, aged from 30 to 90 years. In contrast to our results, Sun et al. (21) showed that human pituitary GH-immunopositive cells decrease during aging. Published results of the study by Sano et al. (22), showed that in 88% of elderly people, mostly men, interstitial perivascular fibrosis was present, which progressed over time and affected the parenchyma of the anterior pituitary gland.

The obtained results showed that the surface area and nuclear-cytoplasmic ratio of immunoreactive LH cells are significantly correlated with the surface area of immunoreactive GH cells. Previous studies have shown that in elderly men, the correlation between morphometric parameters of gonadotropic LH and somatotropic cells is the result of anabolic synergy of GH and androgens under normal physiological conditions (23).

**Conclusion**

A significant correlation between some of the examined morphometric parameters of gonadotropic LH and GH cells indicates the parallel occurrence of adaptation, i.e. compensatory mechanisms, in these cells in men during aging, or their potential mutual interaction during this process, which would be our conclusion.



**Acknowledgements:** This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Number: 451-03-9/2021-14/200113).

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