# THE ROLE OF TESTOSTERONE IN MALE INFERTILITY IN ALBANIA

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***Abstract***

*Infertility is defined as a reproductive disease where the woman cannot get pregnant after 12 months of regular unprotected sex. Globally, infertility affects nearly 15% of reproductive age couples. Even though it affects mostly women, men are risked by it as well, nearly up to 50% of infertility cases. Many factors, including but not limited to underlying diseases, lifestyle, environmental, genetics and age are closely related to damages to semen function. Three hormones are fundamentally related to fertility in males, follicle-stimulating hormone, luteinizing hormone and testosterone. They are kept in balance by a series of checks and supporting pathways, but changes and incorrect signalling leads to hormonal instability resulting in damages to the hypothalamic-pituitary-gonadal axis of the brain. Our study is focused on assessing the levels of these three hormones in males of the Albanian population. We analysed 45 patients over a period of three years from 2020 to 2022 who were randomly selected in the pool and were divided by age groups (from 20-43 years old) to better determine the normal range of hormones in the study and their effects on the male population. We determined that the age group 32-35 in 2020 had the highest peak level of testosterone, decreasing in the following years. The levels of the follicle-stimulating and luteinizing hormones showed no significant changes during the whole three years.*

***Key words*:** *Testosterone, Fertility, Sperm, Level, Males.*

***Përmbledhje***

*Infertiliteti përkufizohet si një sëmundje riprodhuese kur gruaja nuk mund të ngeli shtatzënë pas 12 muajsh seksi të shpeshtë dhe pa mbrojtje. Në nivel global infertiliteti prek rreth 15% të çifteve të moshës riprodhuese. Edhe pse prek më tepër gratë, meshkujt gjithashtu rrezikohen, deri në 50% të rasteve.*

*Shumë faktorë, përfshirë por jo të kufizuar në sëmundjet ekzistuese, stili i jetës, mjedisorë, gjenetikë dhe mosha janë të lidhur ngushtë më dëmtimin në funksionin e spermës. Tre hormone janë ngushtësisht të lidhur me fertilitetin te meshkujt, hormoni folikulo stimulus, luteinizues dhe testosterone. Ata mbahen në balancë nga një seri kontrollesh dhe rrugësh mbështetëse, por ndryshime dhe sinjalizim i gabuar çon në paqëndrueshmëri hormonale që sjell si pasojë dëmtime në aksin hipotalamus- hipofizë- gonadal të trurit. Studimi ynë fokusohet në vlerësimin e nivelit të këtyre tre hormoneve në meshkuj të popullatës shqiptare. U analizuan 45 pacientë për një periudhë kohe tre vjeçare nga 2020 deri në 2022 që u përzgjodhën në mënyrë rastësore dhe u ndanë sipas grupmoshave (nga 20-43 vjeç) për të përcaktuar sa më mirë intervalin normal të hormoneve në studim dhe efektin e tyre në popullatën e meshkujve. U përcaktua që grupmosha 32-35 në vitin 2020 kishte pikun më të lartë të nivelit të testosteronit, që në vitet pasardhëse ka rënë. Niveli i hormonit folikulo-stimulues dhe luteinizues nuk treguan ndryshime sinjifikante gjatë përiudhës tre-vjeçare.*

***Fjalë kyçe:*** *Testosteron, Fertilitet, Spermë, Nivel, Meshkuj*

## Introduction

Male infertility is an ever-increasing medical problem nowadays. Failed reproductive functions in males affect nearly half infertile couples worldwide. An increasing number of factors ranging from environmental, genetics, age and accompanying/underlying diseases lead to abnormal or damaged sperm function. Studies have shown that nearly 15% of couples suffer from infertility and 50% of these cases are attributed to male infertility (Agarwal et al., 2015; Ombelet., 2020; Agarwal et al., 2021; Oud et el., 2022). Even though there are no clear indications of an overall fertility decline in the population, there is significant proof of a decrease in sperm quality (Balawender et al., 2020; Zhou et al., 2022; Garcia-Grau et al., 2022). Male infertility problems can also arise because of low sperm production (oligozoospermia), low sperm mobility (asthenozoospermia), or abnormal sperm morphology (teratozoospermia). According to (Guzick et al 2001), a combination of these factors (oligoasthenoteratozoospermia OAT) is considered to be the most common cause of male infertility. Standardized prevalence of age related to male infertility is reported to be growing 0.3% annually.

Nonetheless, accurate fertility or infertility rates in males represent a whole different challenge for a variety of reasons. The first reason is the lack of surveys targeting males regarding overall medical conditions, and especially

the issue of fertility. The most common target group are either couples, or the female partner of couples who have had problems trying to conceive at a minimum time period of 1 year of unprotected sex.

The second reason is the low number of males undergoing regular medical fertility examinations. This group is extremely small, and generally appears only in the cases when the male presents at a clinic for this specific issue. This leads to the third arising problem, consisting of the underreporting of infertility in males, especially in a patriarchal country (Argwal et al., 2015).

The fourth and last reason regarding male infertility is related to the fact that male infertility is not treated as a disease, leading again to a severe shortage of male statistics. This has been mostly correlated with the lack of a proper definition for infertility and the time frame projections which fertility clinics opt to treat patients, which fall under either the one-year projection category or the three-year projection (Mascarenhas et al., 2012).

This whole process is fundamentally related to the interaction of three hormones (follicle-stimulating hormone, luteinizing hormone and testosterone) operating at a basal level. The role of these hormones has been shown as integral in the spermatogenesis mechanism of action as shown in figure 1:

Spermatogenesis is initiated and regulated by the hypothalamus-pituitary-gonadal axis. Gonadotropin-releasing hormone (GnRH) is secreted as synchronized pulses by the nerve endings and acts on the pituitary gland to stimulate the secretion of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). FSH operates on the Sertoli cells, whereas LH interacts on the Leydig cells. The importance of FSH in spermatogenesis is questionable (Moudgal and Sairam., 1998; Plant and Marshall., 2001), but is necessary for the activation of the spermatogenesis process.

Embryo cells do not have androgen receptors, and because of that the secreted androgens from the Leydig cells interact by using the Sertoli cells receptors as intermediaries. The secreted testosterone from the Leydig cells coupled with the inhibin secreted form the Sertoli cells and the oestradiol formed by the aromatization of testosterone operate on the hypothalamus and pituitary gland to regulate gonadotropin secretion via a negative feedback mechanism. This whole mechanism shows the importance of the GnRH, FSH, LH and testosterone levels in the overall normal spermatogenesis.

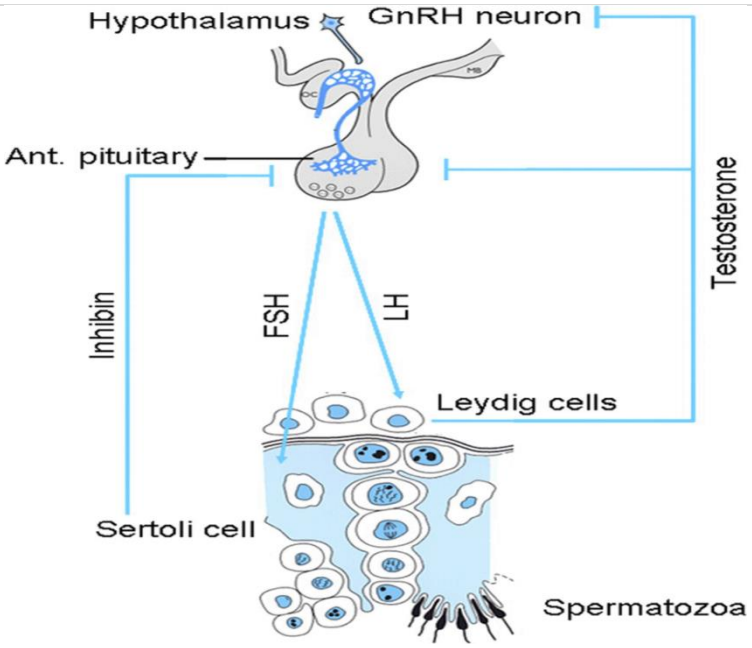


**Figure 1:** Spermatogenesis process

Hormonal unbalance in males affecting fertility is very similar to the natural old age symptoms, leading to an increased difficulty in diagnosing the patient. Symptoms include, but are not limited to: thinning or loss of hair, weight gain/loss, low sexual desire or erectile dysfunction, fatigue, depression and poor sperm parameters (low sperm count, low mobility and abnormal morphology).

In our study we wanted to show an overview of the matter of infertility in Albania and the underlying hormones affecting males and their shift in the different age groups, because of all the aforementioned reasons.

## Materials and methods

45 male patients were analyzed for a three-year period (2020-2022). Samples are randomly chosen from the pool of patients who are diagnosed at the Intermedica central laboratory. All the samples are analyzed according to State regulations and specifications. Blood samples are collected from the patient and given a unique 9-digit barcode. Serum is put into standard sampling tubes containing Li-heparina, K2-EDTA and K3-EDTA plasm. The samples are stable for 5 days at a temperature of 20-25°C, 14 days at 2-8°C, and for 6 months at ‑20°C (± 5°C). Thaw only once.

Samples are centrifuged for 10 minutes at 4000rpm in a Cobas machine to collect the serum for further analysis.

Three different hormone levels are analyzed and measured according to the standardized protocol:

- Follicle-stimulating hormone (FSH):

Analyzed with the electrochemiluminescence immunoassay “ECLIA” for a total time of 18 minutes.

First incubation of 40 µL sample comprised of a specific monoclonal biotinylated FSH antibody, coupled with a specific monoclonal ruthenium FSH antibody to form a complex sandwich.

Second incubation consists of adding streptavidin-coated nanoparticles to adhere to the sandwich via biotin and streptavidin. The nanoparticles magnetically interact with an electrode. Interaction on the electrode leads to a chemiluminescence emission readable by a photomultiplier.

- **Luteinizing hormone (LH)**

Same procedure as FSH, but with a volume of 20 µL in the samples, instead of 40 µL.

- **Testosterone**

Same procedure as FSH, but with a volume of 12 µL in the samples, instead of 40 µL.

Reference values for testosterone are: 2.8-8 ng/mL> 18 years-old;

Reference values for LH are: 1.7-8.6 mIU/mL;

Reference values for FSH are: 1.5-12.4 mIU/ mL;

**Results and discussion**

The number of male patients randomly selected for this study was 45 for each year (2020, 2021 and 2022) and analyzed for all three hormones. Patients age ranges from 23 years old to 43 years old, considered to be the most prolific years for reproduction, peaking at 25-29 years old.

As shown in figure 2, we see a slight increase in the mean values of testosterone levels in our sample population. In 2020 the mean values of testosterone are 4.2ng/mL, whereas in 2021 these values go up to nearly 4.7ng/mL and just 4.8ng/mL in 2022. Even taking into consideration the low

values in 2020, nonetheless the mean levels of testosterone fall into the reference values of 2.8-8ng/mL. These changes could be attributed to a number of factors: the younger population is reaching peak fertility maturity. Even though the older patients are getting older, this could lead to a decrease in sperm quality (beginning at around 30-32 years old) and quantity (significant decrease at 45 and older), but not necessarily affecting or being affected by testosterone.

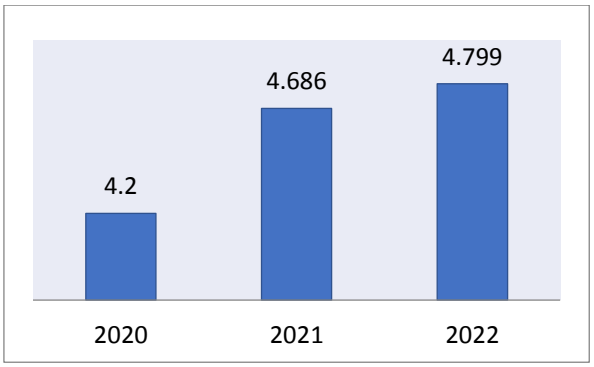


**Figure 2:** Mean levels of testosterone in male population. Units are in ng/mL

Another factor to take into consideration is the end of the pandemic, leading to lifestyle changes in the male population varying from increased physical activity and intensity, access to better food quality, general mood improvement and better mental health. Several studies have shown that usage of narcotic substances, alcohol consumption and in many cases the type of job you have, can affect testosterone levels (Duca et al., 2019; David and Kirby 2022; Koh et al., 2022; Smith et al., 2023). Smoking is another factor to be taken into account, but studies have shown conflicting results; there are studies that support the idea that smoking lowers testosterone levels (El Salam et al., 2021) and other studies that show that tobacco consumption increases them (Svartberg and Jorde., 2007; Zhao et al., 2016).

This has been proven by Zhao et al., 2016 that shows that smoking leads to an increase of testosterone levels since nicotine metabolites and androgens have the same mechanism of discarding and nicotine has a higher affinity, potentially blocking androgen ejection. A high level of testosterone nonetheless is not necessarily affiliated with male fertility. A study by Tang et al., in 2019 shows a high level of testosterone in heavy smokers, but a significant decrease in sperm volume and total sperm count, but no significant quality in the semen. This study is supported by the study from Osadchuk et

al., 2023, which showed that smokers from a population of 1222 males, heavy smokers showed a significant decrease in semen volume, total sperm count, motility and concentration and an increase in semen teratozoospermia and DNA fragmentation. Sedentary lifestyle, with ever increasing time spent sitting down has also led to lower levels of testosterone.

Testosterone levels are also afflicted by stress. A study by Afrisham et al., 2016 showed significant low levels of salivary testosterone in males under stress conditions, whereas female participants had notable, but not significant lower levels of testosterone under the same conditions of acute stress. And the study by Xiong et al in 2022 showed that male rats under chronic stress conditions for 3 weeks showed a significant decrease in bodyweight and genital index, as well as serum testosterone levels.

Reference values of testosterone and mean levels seen in figure 2, show that the situation in our study group comprised only of Albanian males, is within all parameters. The real story is that there are multiple cases when in fact that is not factually true. As shown in figure 3, these values vary quite drastically, differing a lot throughout the years. 2020 is the year with both values at its lowest (8.61 and 0.9ng/mL, max and min respectively), peaking in 2021 (~11.1 and 1,8ng/mL) and then showing a slight decrease in 2022 (9.74 and 1.43ng/mL). There are two main factors to be considered here:

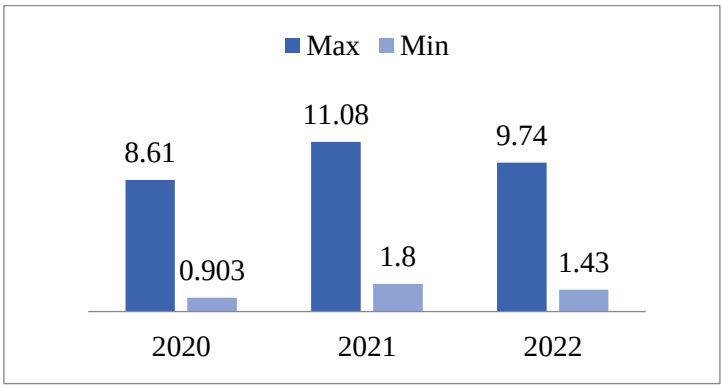


**Figure 3:** Maximal and minimal values for testosterone

1) The Covid-19 pandemic in Albania sent the nation to a total shutdown and lockdown of people, which in turn lead to overeating, increased obesity and more sedentary lifestyle, which results in low testosterone. This also coupled with an ever-decreasing mental health and accumulated stress. The

return of life to a pre-pandemic situation considerably improved the parameters of persons in general, including testosterone levels.

2) The males in the study group are usually persons already suffering from a reproductive anomaly, usually low testosterone levels or affiliated hormonal unbalance and as such as being treated with prescription drugs to increase testosterone levels. Taking this factor into account, it would result in the artificial increase of the mean levels of testosterone of patients.

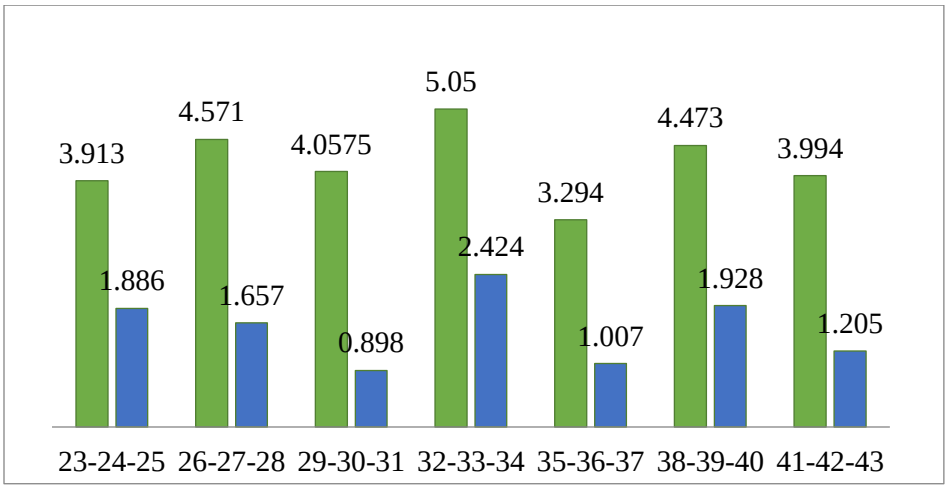


**Figure 4:** Mean values (green) and std deviation (blue) for males in 2020

Figure 4 shows the ongoing trend of testosterone levels worldwide, peaking around 25-29 and starting to get lower with each passing group. Even though there is a deviation in the study group, which has also been reported by the Albania Institute of Statistics in 2021 (citation missing) that males in Albania are predominantly starting families after the age of 30, which correlates with our findings as well. It has become practice for new couples to undergo a series of tests and treatments before starting a new family, which in Albania has drastically shifted after the age of 30, peaking between 32-35 years old.

No more than a decade ago, this trend was predominantly shifted before both partners hit 30 years old, usually in between 25-26 for the female partner, and 28-30 for the male counterpart. What is also of note in the figure is the absence of 18–22-year-old young adult males. One key element is that biologically this age group is part of the low-risk group for low testosterone levels. And the next major feature, is the lack of information and awareness campaigns focusing on said issue. A simple survey of the patients when going to the clinic regarding testosterone levels during the 2020 pandemic showed clear results:

since people are stuck at home and have too much free time, it has led to more frequent and regular sexual relations between partners, which in turn has boosted testosterone levels.

Figure 5 shows the mean values and std deviation for testosterone in males in 2021. Compared to the previous year a left higher shift is seen in males younger than 32, peaking in the age group 29-31. There is a significant increase in all other younger groups as well, and a slight decrease in males 32 and older which is attributed to the biological lowering levels of testosterone. What is notable are the significant diminished values in the 32-35 age group, which is attributed to the birth of newborns and the lack of intimate relations in the couple, apart from the aforementioned factors.

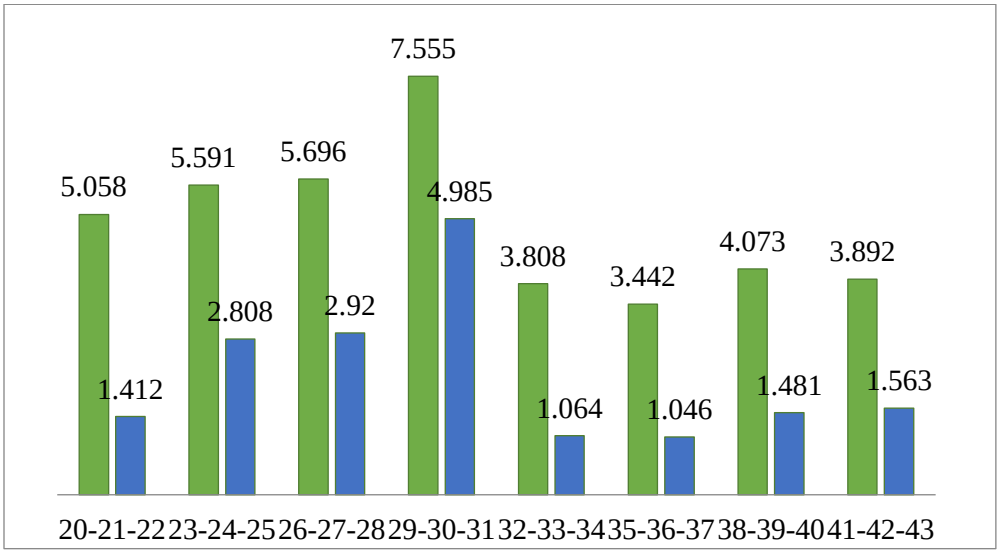


**Figure 5:** Mean values (green) and std deviation (blue) for males in 2021

In figure 6 we see an expected trend of decreased testosterone values, compared to the year 2021 because the pandemic was over and life had returned to its normalcy. The return of people in the office means a more inactive life and less physical activity. The increased consumption of alcohol, tobacco, and recreational drugs coupled with a sedentary lifestyle would lead to a lower libido and sexual drive, resulting in decreased levels of testosterone as supported by the data.

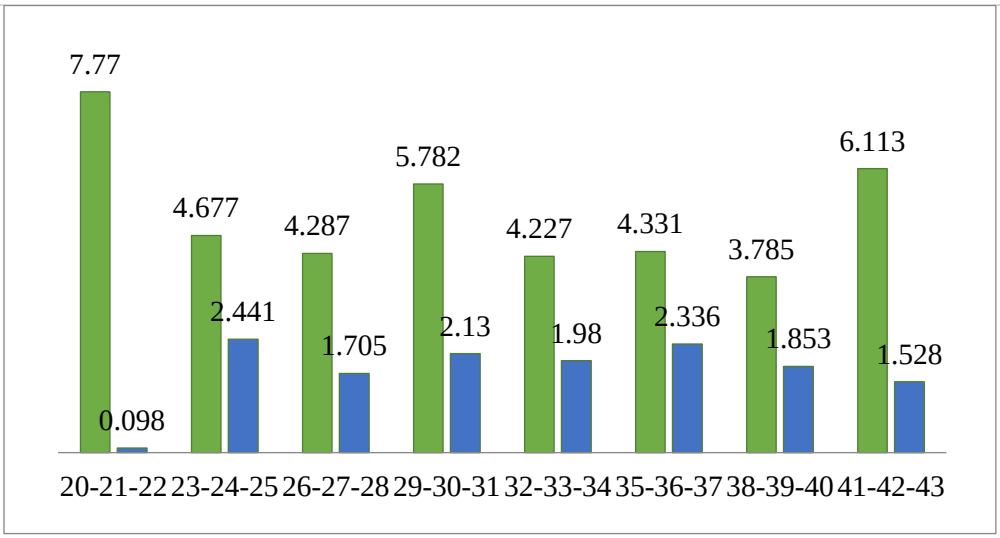


**Figure 6:** Mean values (green) and std deviation (blue) for males in 2022

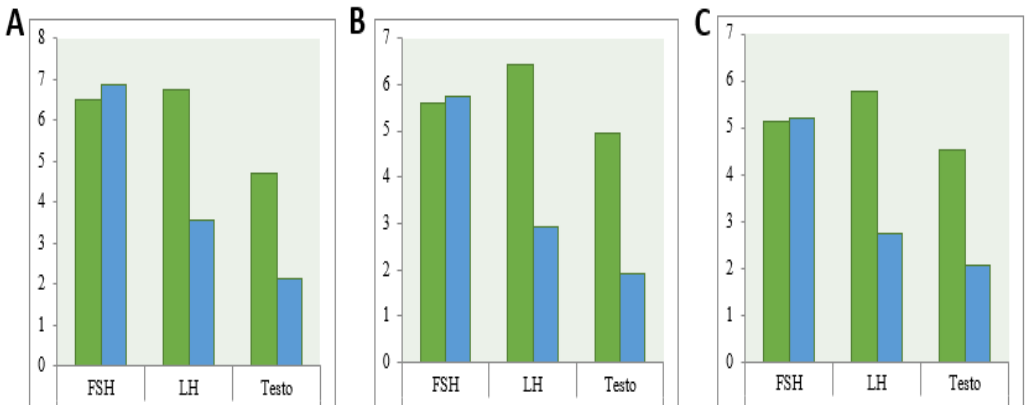


**Figure 7:** Mean levels (green) and std deviation (blue) of FSH, LH and Testosterone in A- 2020; B- 2021; C- 2022

The next analysis for the follicle-stimulating hormone and luteinizing hormone showed no significant changes in all three years of the study. Both FSH and LH fall between the ranges of the reference values, leading to the conclusion that spermatogenesis is functioning normally. In the case when FSH levels would be higher, then sperm production would be outside normal parameters and if LH levels are high, they would directly impact synthesis of

testosterone. High levels of LH could also be used as a marker to determine damage or failure of the testis.

Abnormal spermatogenesis in the testes would correlate with a high stimulated pituitary gland and overproduction of LH and FSH and low levels of testosterone. Testosterone by itself is not directly linked to the production of semen, but its low serum levels affect normal penile erection and lower libido, with the outcome being higher infertility rates.

## Conclusions

In conclusion, our study is focused on an area where findings in Albania are scarce and the data is either lacking or underreported. We elucidated the indirect importance of testosterone in male fertility and showed findings about the situation in our country which are easily obtainable with simple blood work, relatively cheap and with long lasting and preventive effects. Even though the sample group is still small, this could help as a stepping stone for a more comprehensive and detailed assay of the Albanian male population.

## Acknowledgements

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### *References*

Afrisham R, Sadegh-Nejadi S, SoliemaniFar O, Kooti W, Ashtary-Larky D, Alamiri F, Aberomand M, Najjar-Asl S, Khaneh-Keshi A. Salivary Testosterone Levels Under Psychological Stress and Its Relationship with Rumination and Five Personality Traits in Medical Students. Psychiatry Investig. 2016 Nov;13(6):637-643. doi: 10.4306/pi.2016.13.6.637. Epub 2016 Nov 24. PMID: 27909455; PMCID: PMC5128352.

Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015 Apr 26;13:37. doi: 10.1186/s12958-015-0032-1. PMID: 25928197; PMCID: PMC4424520.

Ashok Agarwal, Saradha Baskaran, Neel Parekh, Chak-Lam Cho, Ralf Henkel, Sarah Vij, Mohamed Arafa, Manesh Kumar Panner Selvam, Rupin Shah, Male infertility, The Lancet, Volume 397, Issue 10271, 2021, Pages 319-333, ISSN 0140-6736, https://doi.org/10.1016/S0140-6736(20)32667-2.

Balawender K, Orkisz S. The impact of selected modifiable lifestyle factors on male fertility in the modern world. Cent European J Urol. 2020;73(4):563-568. doi: 10.5173/ceju.2020.1975. Epub 2020 Oct 10. PMID: 33552585; PMCID: PMC7848840.

David, J. and Kirby, M. (2022), Testosterone deficiency, the unrecognised consequence of the opioid epidemic in men. Trends Urology & Men Health, 13: 22-27. https://doi.org/10.1002/tre.846

Duca Y, Aversa A, Condorelli RA, Calogero AE, La Vignera S. Substance Abuse and Male Hypogonadism. J Clin Med. 2019 May 22;8(5):732. doi: 10.3390/jcm8050732. PMID: 31121993; PMCID: PMC6571549.

El Salam, Mohamed A.A.a; Zaki, Shadya; Mousa, Mohamed S.b,; Motawi, Ahmeda. Effect of cigarette smoking on serum testosterone level among male smokers: a cross-sectional study. The Egyptian Journal of Chest Diseases and Tuberculosis 70(1):p 124-127, Jan–Mar 2021. | DOI: 10.4103/ejcdt.ejcdt_61_20

Garcia-Grau E, Lleberia J, Costa L, Guitart M, Yeste M, Benet J, Amengual MJ, Ribas-Maynou J. Decline of Sperm Quality over the Last Two Decades in the South of Europe: A Retrospective Study in Infertile Patients. Biology (Basel). 2022 Dec 30;12(1):70. doi: 10.3390/biology12010070. PMID: 36671762; PMCID: PMC9856056.

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D, Vogel DL; National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001 Nov 8;345(19):1388-93. doi: 10.1056/NEJMoa003005. PMID: 11794171.

Jie Zhao, June Yue Yan Leung, Shi Lin Lin, C. Mary Schooling, Cigarette smoking and testosterone in men and women: A systematic review and meta-analysis of observational studies, Preventive Medicine, Volume 85, 2016, Pages 1-10, ISSN 0091-7435, https://doi.org/10.1016/j.ypmed.2015.12.021.

Koh K, Kim SS, Kim JS, Jung JG, Yoon SJ, Suh WY, Kim HG, Kim N. Relationship between Alcohol Consumption and Testosterone Deficiency according to Facial Flushes among Middle-Aged and Older Korean Men. Korean J Fam Med. 2022 Nov;43(6):381-387. doi: 10.4082/kjfm.21.0173. Epub 2022 Nov 20. PMID: 36444123; PMCID: PMC9708857.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9:e1001356. doi: 10.1371/journal.pmed.1001356.

Moudgal NR, Sairam MR. Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates? Hum Reprod. 1998 Apr;13(4):916-9. doi: 10.1093/humrep/13.4.916. PMID: 9619548.

Ombelet W. WHO fact sheet on infertility gives hope to millions of infertile couples worldwide. Facts Views Vis Obgyn. 2020 Jan 8;12(4):249-251. PMID: 33575673; PMCID: PMC7863696.

Osadchuk L, Kleshchev M and Osadchuk A (2023) Effects of cigarette smoking on semen quality, reproductive hormone levels, metabolic profile, zinc and sperm DNA fragmentation in men: results from a population-based study. *Front. Endocrinol.* 14:1255304. doi: 10.3389/fendo.2023.1255304

Oud, M.S., Smits, R.M., Smith, H.E. *et al.* A de novo paradigm for male infertility. *Nat Commun* **13**, 154 (2022). https://doi.org/10.1038/s41467-021-27132-8

Plant, T.M. and Marshall, G.R. (2001) The Functional Significance of FSH in Spermatogenesis and the Control of Its Secretion in Male Primates. Endocrine Reviews, 22, 764-786. http://dx.doi.org/10.1210/edrv.22.6.0446

Smith, S. J., Lopresti, A. L., & Fairchild, T. J. (2023). The effects of alcohol on testosterone synthesis in men: a review. *Expert Review of Endocrinology & Metabolism*, *18*(2), 155–166. https://doi.org/10.1080/17446651.2023.2184797

Svartberg J, Jorde R. Endogenous testosterone levels and smoking in men. The fifth Tromsø study. Int J Androl. 2007 Jun;30(3):137-43. doi: 10.1111/j.1365-2605.2006.00720.x. Epub 2006 Nov 27. PMID: 17163954.

Tang Q, Pan F, Wu X, Nichols CE, Wang X, Xia Y, London SJ, Wu W. Semen quality and cigarette smoking in a cohort of healthy fertile men. Environ Epidemiol. 2019 Aug 13;3(4):e055. doi: 10.1097/EE9.0000000000000055. PMID: 31538136; PMCID: PMC6693933.

Xiong X, Wu Q, Zhang L, et al. Chronic stress inhibits testosterone synthesis in Leydig cells through mitochondrial damage via Atp5a1. *J Cell Mol Med*. 2022; 26: 354–363. doi:10.1111/jcmm.17085

Zhou M, Yao T, Li J, Hui H, Fan W, Guan Y, Zhang A and Xu B (2022) Preliminary prediction of semen quality based on modifiable lifestyle factors by using the XGBoost algorithm. *Front. Med.* 9:811890. doi: 10.3389/fmed.2022.811890