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# The correlation of intratesticular testosterone with body mass index and smoking.

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ABSTRACT

Intratesticular testosterone plays a crucial role in male reproductive function and overall health. Body Mass Index (BMI) and smoking are known lifestyle factors that can influence hormonal balances. Elevated BMI has been associated with alterations in testosterone levels, impacting male fertility and endocrine function. The aim was to examine the concurrent relationships between intratesticular testosterone, BMI, and smoking habits. Biopsies of the testicles were taken from 32 patients with non-obstructive azoospermia. Testicular sperm extraction (TESE) under spinal or general anesthesia was performed after a comprehensive history, physical examination, and investigations. After performing a minced biopsy and centrifuging the fluid, testicular fluid was extracted. The highest intratesticular testosterone levels were in normal patients and lowest levels in over weight patient; on the contrary the highest serum 17-hydroxyprogesterone levels were found in over weight patient and lowest levels in normal weight patients; There were insignificantly higher both intratesticular testosterone and 17-hydroxyprogesterone levels in non-smokers. In conclusion, the statistical analysis revealed that there is an insignificantly low correlation between intratesticular testosterone levels, BMI, and smoking.

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**KEYWORD**

Azoospermia, BMI, Smoking, Intratesticular Testosterone.

## 1. Introduction

The adult testis has two main functions: producing spermatozoa and releasing testosterone (T) (Mclachlan R I et al., 2002 [1]). It is well-established that testosterone is crucial for both the initiation and maintenance of the spermatogenic process (Patel A. et al., 2019 [2]). In response to luteinizing hormone (LH), Leydig cells in the testicular interstitium produce and secrete this hormone (Smith L B and Walker WH 2014 [3]). Leydig cells, Sertoli cells, and peritubular cells (but not developing germ cells) contain the androgen receptor, which is how intratesticular testosterone (ITT) exerts its effects (Bremner W J et al., 1994 [4]). When exogenous testosterone is used in experiments to block pituitary LH secretion, the resulting decreases in ITT and sperm production are striking (Matthiesson KL et al., 2005 [5]). However, in men with hypogonadotropic hypogonadism due to pituitary disease, administration of the LH receptor agonist human chorionic gonadotropin (HCG) increases ITT and stimulates spermatogenesis (Finkel D M et al., 2010 [6]). Hypogonadotropic, hyperestrogenic hypogonadism results from central and peripheral changes in the hypothalamic-pituitary-gonadal axis brought on by obesity. The levels of testosterone and inflammation are controlled by adipose tissue-derived factors such as leptin and adipokines. Fragmentation of sperm DNA and an increase in reactive oxygen species are both effects of systemic inflammation. Spermatogenesis is hindered by an elevated testicular temperature brought on by a sedentary lifestyle and poor eating habits. Comorbid conditions may account for the varying degrees to which obesity affects hormone levels, semen parameters, sperm DNA integrity, and pregnancy rates. Weight loss as a treatment has also yielded mixed results (Kahn BE and Brannigan RE., 2017 [7]).

Tobacco use is widely recognized as a global epidemic, impacting both high- and low-income countries due to the wide range of health problems it is known to cause. The risk of hypogonadism may also be increased by smoking, which has been linked to a decrease in the function of several endocrine glands, including the gonads (El Salam T F N et al., 2020 [8]).

The aim of this study is to investigate the correlation between intratesticular testosterone levels, Body Mass Index (BMI), and smoking habits in a cross-sectional context. By examining these associations, the research aims to contribute insights into the potential impact of lifestyle factors on intratesticular testosterone; for this cross-sectional study, data was collected, organized, and entered into using IBM SPSS V26 to be analyzed and create a descriptive study. Tables, graphs, and calculations were done using IBM SPSS V26.

## *2. Patients and Methods*

Thirty-two infertile males were enrolled in the present cross-sectional study. The results were expressed in mean plus minus standard deviation. The mean patients' age was 33.06 ± 5.91, with the mean duration of infertility equal to 8.06 ± 5.1 years. The mean body mass indices were 28.32 ± 3.26; patients were ranked according to body mass indices into 6 (18.8%) normal-weight patients, 18 (56.2 %) over-weight patients, and 8 (25.0%) obese patients. Fourteen (43.8%) males were smokers, and 18 (56.2%) were non-smokers.

This research is a prospective cross-sectional study that will be conducted between November 1, 2022, and June 1, 2023, at the Institute for Infertility Diagnosis and Assisted Reproductive Technologies. Verbal consent was taken from all patients; the study was approved by the ethical committee of the Institute for Infertility Diagnosis

and Assisted Reproductive Technologies/University of Nahrain.

Biopsies of the testicles were taken from 32 patients with non-obstructive azoospermia. Testicular sperm extraction (TESE) under spinal or general anesthesia was performed after a comprehensive history, physical examination, and investigations. After performing a minced biopsy and centrifuging the fluid, testicular fluid was extracted. In addition, blood samples were collected from all patients to measure serum testosterone.

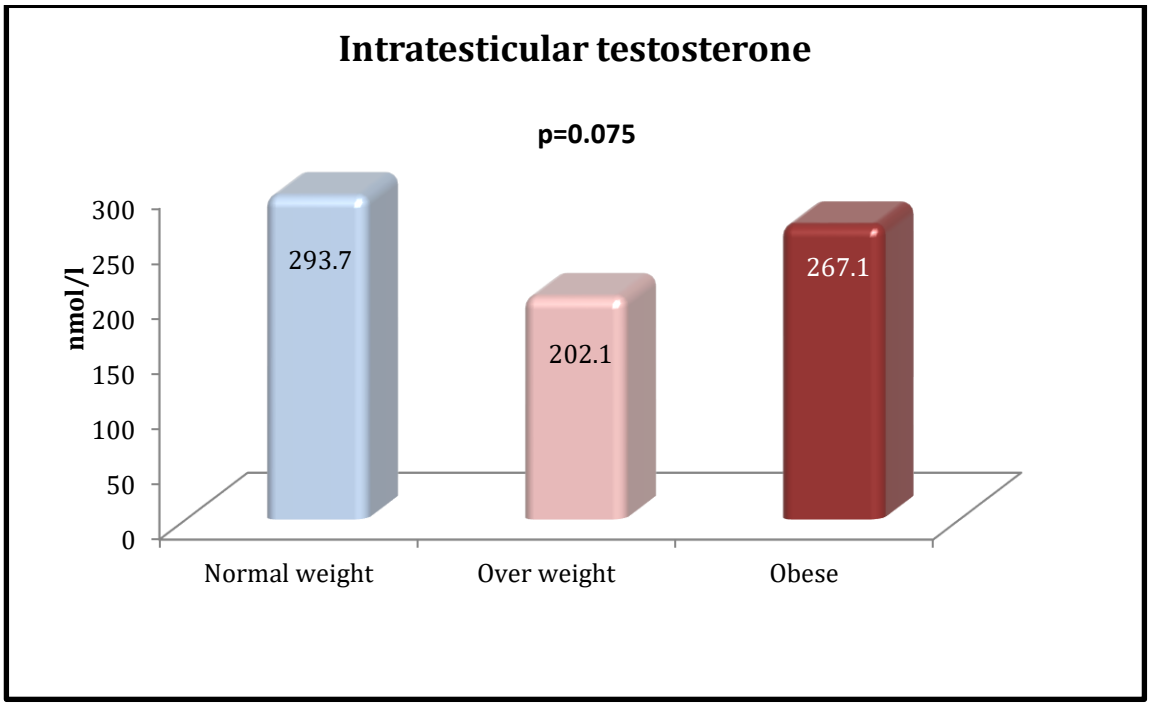
The testicular fluid was taken after mincing the testicular tissue. The fluid was put in an Eppendorf tube, and it was centrifuged at 3000 rpm for 10 minutes at -40 °C. The pellet was discarded, and the supernatant was kept. All samples were stored in a deep freezer at -70 °C, and the intratesticular testosterone was analyzed through the enzyme-linked immunosorbent assay (ELISA) technique. Serum 17-hydroxyprogesterone was analyzed through the ELISA technique. The kit was purchased from Snibe and labeled "Maglumi 17-OH progesterone (CLIA) ELISA Kit. Blood samples were taken from a vein at the antecubital fossa after fully sterilizing the area. The blood was put in a gel tube. The serum was extracted by centrifugation at 3000 rpm for 10 minutes

## *3. Results*

There were no significantly differences regarding intratesticular testosterone ($p$=0.075) and serum 17-hydroxyprogesterone ($p$=0.581) levels between normal weight, overweight, and obese patients. The highest intratesticular testosterone levels were in normal patients, and the lowest levels in the overweight patient (293.7 ± 14.8 vs. 202.1 ± 27.07, respectively); on the contrary, the highest serum 17-hydroxyprogesterone levels were found in the overweight patient and lowest levels in normal weight patients (2.02 ± 0.19 vs. 2.44 ± 0.24 respectively) as demonstrated in Figure (1)

There were insignificantly higher both intratesticular testosterone and 17-hydroxyprogesterone levels in non-smokers (241.3± 18.3 vs. 228.1 ± 32.4; $p$=0.781) and (2.41 ± 0.16 vs. 2.22 ± 0.15; $p$=0.539) respectively as presented in table 1.

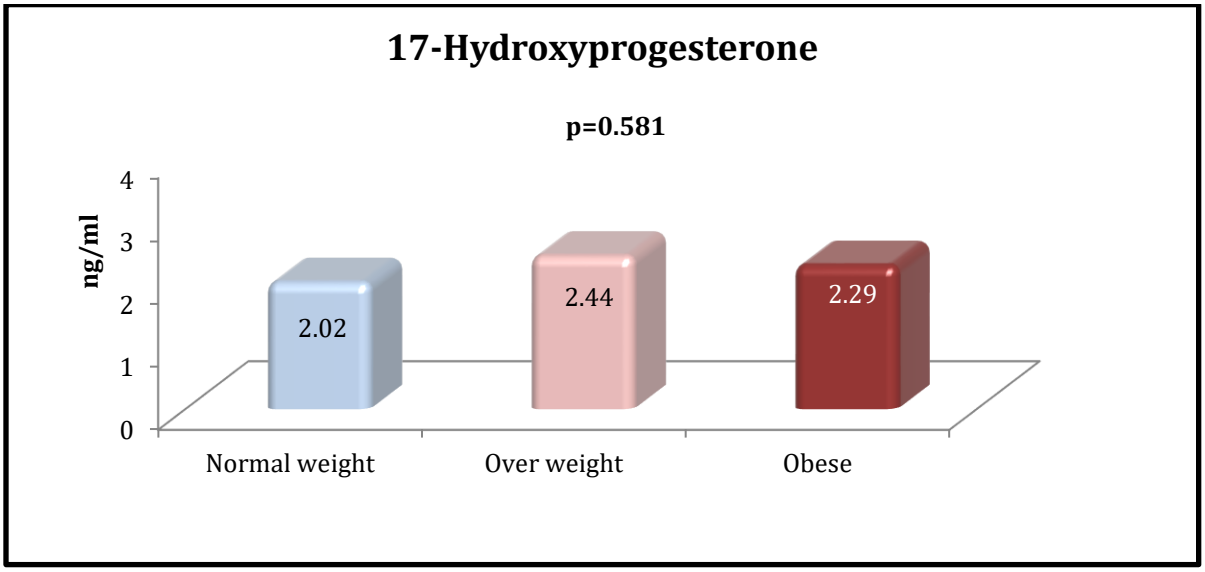
**Figure 1:** Comparison of intratesticular testosterone levels between normal, overweight and obese patients



***(Table 1):*** Comparison of intratesticular testosterone and serum 17 hydroxyprogesterone levels between smokers and non-smokers

| Parameters | Smokers N.=14 | Non-Smokers N.=18 | p-value |
|---|---|---|---|
| Intratesticular testosterone (nmol/l) | 228.1 ± 32.4 | 241.3 ± 18.3 | 0.781 Ŧ NS |
| 17-Hydroxyprogesterone (ng/ml) | 2.22 ± 0.15 | 2.41 ± 0.16 | 0.539 Ŧ NS |

NS: Not significant (p> 0.05); Ŧ: Independent sample t-test




***(Figure 2):*** Comparison of 17-hydroxyprogesterone levels between normal, overweight and obese patients

## 4. Discussion

The present study found that intratesticular testosterone and 17-hydroxyprogesterone were insignificantly affected by BMI. On the contrary, (Lima et al., 2022 [9]) concluded that obesity and aging negatively affected 17-OHP independent of LH, so they suggested that obesity may have a direct impact on testicular function rather than an indirect effect via a decline in pituitary function, as evidenced by changes in serum T and 17-OHP (ITT). One possible explanation for these patients' low testosterone levels and infertility is a decrease in ITT associated with their obesity, as mentioned by (Katib A., 2015 [10]). Another epidemiological study by (Lokeshwar et al., 2021 [11]) showed that obesity is correlated with lower levels of all three forms of testosterone (T): free testosterone (FT) and bioavailable testosterone (BT). Multiple studies have found a statistically significant negative association between T and obesity (Hackett et al., 2013 [12]).

This difference could be due to an increase in BMI, which is not always an indication of obesity; that is an increase in weight (BMI) because of an increase in muscular building, so future studies should evaluate the effects of leptin on ITT and 17-OHP, as was also suggested by (Lima et al., 2022 [9]).

Smoking is associated with numerous health risks, including a large number of non-communicable diseases, and is widely regarded as an epidemic throughout the world, affecting both developed and developing nations. Smoking has also been linked to a reduction in the function of several endocrine glands, including the gonads, which may increase the risk of hypogonadism (El Salam et al., 2021 [8]) The results of the present study showed insignificantly higher intratesticular testosterone and 17-hydroxyprogesterone levels in non-smokers than smokers; the statistical

insignificance of the results may be due to the small group of patients included in the study. There are a number of hypotheses that have been proposed regarding the mechanisms by which smoking lowers testosterone levels. This is due to Leydig cell dysfunction, which can be caused by a variety of factors, including chronic hypoxia, smoke-induced oxidative stress (ROS), the neuroendocrinal effect of nicotine on the hypothalamic-pituitary-gonadal axis, and a direct cytotoxic effect on Leydig cells, which can either cause downregulation of key enzymes of steroidogenesis (NR5A1, CYP11A1, and 3b-HSD1). Or by inducing apoptosis by increasing the expression of proapoptotic proteins like Bax and caspase-3 while simultaneously decreasing the expression of antiapoptotic proteins like Bcl-2 (El Salam et al., 2021 [8]; Kizilkan et al., 2019 [13]; Pavan-Jukic et al., 2020 [14]) observed that smoking status could indicate a potential factor for negative sperm retrieval ($p = 0.038$). Only 4 out of 15 smokers (26.7%) were TESE-positive.

## *6- Conclusion*

In conclusion, the statistical analysis of this cross-sectional study revealed that there is an insignificantly low correlation between intratesticular testosterone levels, BMI, and smoking. The findings suggest that, within the limitations of this study, variations in BMI and smoking habits do not appear to be robust predictors of intratesticular testosterone concentrations. It is important to acknowledge the constraints of a cross-sectional design and consider the need for further longitudinal investigations to comprehensively explore the complex dynamics of hormonal regulation in relation to lifestyle factors**.**

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***Author Contribution***

Sara Hamdi Salih performed the study, and Ali Ibrahim Rahim Ula Al-Kawaz supervised the work .

***Conflict of Interest***

The authors declare no conflict of interest .

***Ethical Clearance***

The study was approved by the Ethical Approval Committee.

***Financial Disclosure***

There is no financial disclosure.

***Ethical Clearance***

The study was approved by the Ethical Approval Committee

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