

Hormones FSH, LH and Inhibin B levels in adolescent treated and followed due to varicocele at pediatric surgery Split

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**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

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**HORMONES FSH, LH, AND INHIBIN B LEVELS IN ADOLESCENTS TREATED
AND FOLLOWED DUE TO VARICOCELE AT PEDIATRIC SURGERY SPLIT**

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To my Fabien, forever and always.*

LIST OF BBREVIATIONS

ASMR – American Society for Reproductive Medicine

AUA – American Urological Association

CDUS – Color Doppler ultrasonography

EAU – European Association of Urology

ESPU – European Society for Pediatric Urology

FSH – Follicle-stimulating hormones

GnRH – Gonadotropin-releasing hormones

LH – Luteinizing hormone

SA – Semen analysis

TMC – Total mobile count

VR – Varicocele repair

WHO – World Health Organization

1. INTRODUCTION

1.1. ANATOMY OF THE MALE REPRODUCTIVE SYSTEM

The male reproductive system is made up of the scrotum, penis, testes, epididymis, vas deferens, and the prostate, as illustrated in Figure 1. These structures are significantly supplied with blood vessels and contain numerous glands and ducts. Their main function is to promote the production, storage, and release of sperm, facilitating fertilization. They are also responsible to produce important androgens for male development (2).

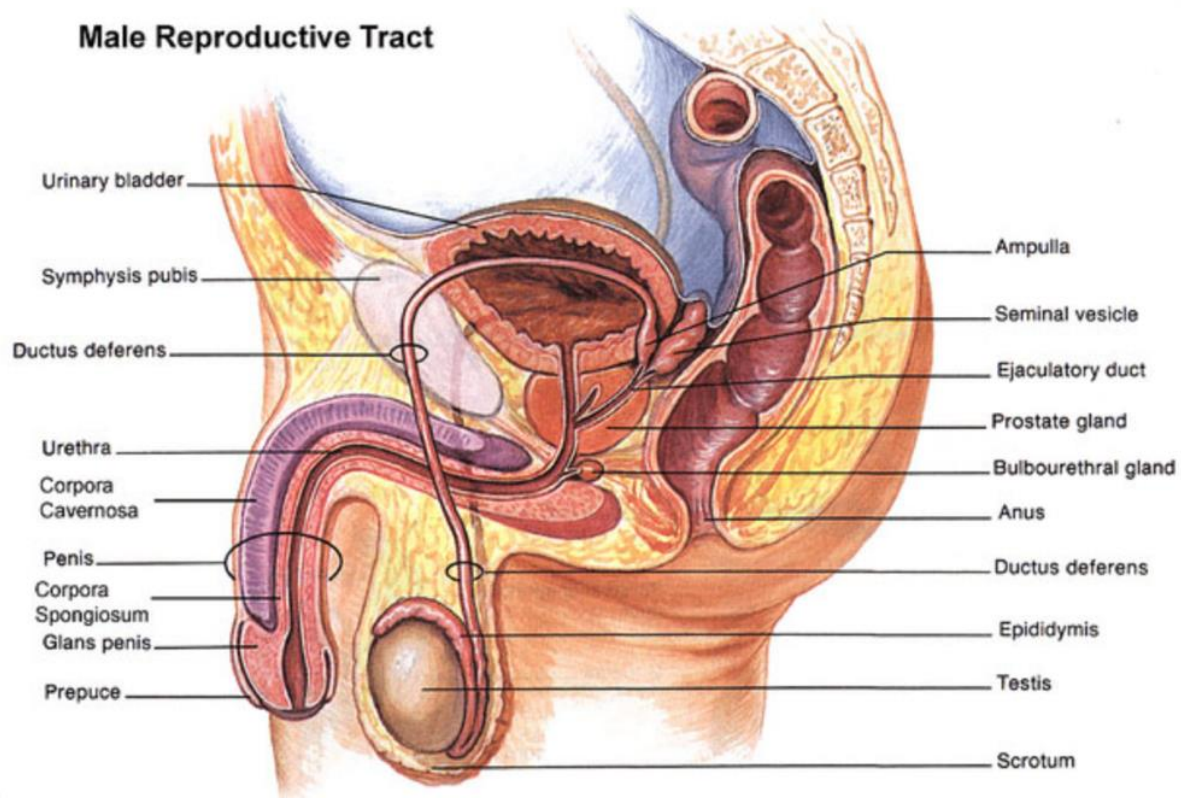


Figure 1. Anatomy of the male reproductive system (1).

1.1.1. SPERMATIC CORD

The spermatic cord contains various structures that extend to and from the testis. This cord-like structure is responsible for suspending the testis within the scrotum. Originating at the deep inguinal ring lateral to the inferior epigastric vessels, the spermatic cord travels through the inguinal canal, emerges at the superficial inguinal ring, and ends at the posterior border of

the testis in the scrotum (2). During prenatal development, the spermatic cord is enveloped by fascial coverings derived from the anterolateral abdominal wall. These coverings are sequentially layered. The innermost layer is the internal spermatic fascia, followed by the cremasteric fascia; and finally, the external spermatic fascia, which constitutes the outermost layer. Within the cremaster fascia, one can find loops of the cremaster muscle. This muscle is composed of the lowermost fascicles of the internal oblique muscle, which originate from the inguinal ligament.

In response to cold, the cremaster muscle is responsible for reflexively pulling the testis upwards within the scrotum (3). However, in a warm environment, the cremaster relaxes causing the testis to descend deeply in the scrotum. Both these reflexes aim to maintain the optimal temperature of the testis, which is crucial for spermatogenesis. This optimal temperature is approximately one degree cooler than the body's core temperature. The genital branch of the genitofemoral nerve is responsible for innervating the cremaster muscle. Within the spermatic cord, various structures can be identified, including the following: ductus deferens, testicular artery, artery of ductus deferens, cremasteric artery, pampiniform venous plexus, sympathetic nerve fibers, the genital branch of the genitofemoral nerve, lymphatic vessels, and vestige of processus vaginalis (2).

1.2.2. TESTES

The testes, or testicles, functioning as male gonads, are paired oval-shaped reproductive glands responsible to produce sperm, also known as spermatozoa. In addition, they produce male hormones primarily testosterone. Suspended within the scrotum by the spermatic cords, the position of the left testis is typically slightly lower than the right testis (4). Each testis is enveloped by the visceral layer of the tunica vaginalis, apart from the segments where the testis connects to the epididymis and the spermatic cord. Located next to the internal spermatic fascia, the parietal layer of the tunica vaginalis is more extensive than the visceral layer. It extends upwards slightly onto the distal part of the spermatic cord. A small quantity of fluid found within the tunica vaginalis separates the visceral and parietal layers, providing the testis freedom to move within the scrotum (2). Located within the testes are found the seminiferous tubules in which the spermatozoa are produced.

The testicular arteries, which extend along the spermatic cord supply the testes, and a branch of these arteries establishes an anastomosis with the artery of the ductus deferens. An important anatomical structure to consider when discussing varicocele is the pampiniform

venous plexus, originating from the testes and epididymis. This plexus is a network of 8-12 veins situated anteriorly to the ductus deferens and surrounding the testicular artery in the spermatic cord. Together with the cremasteric and dartos muscles, it contributes to the testis thermoregulation system, keeping the gland at a consistent temperature. The pampiniform plexus's veins converge superiorly, leading to the formation of the right testicular vein, which enters the inferior vena cava (IVC), and the left testicular vein, which merges into the left renal vein (2, 4).

1.1.3. EPIDIDYMIS AND DUCTUS DEFERENS

On the posterior surface of the testis, an elongated structure can be found, the epididymis. The efferent ductules in the testis are responsible for transferring newly produced sperm from the rete testis to the epididymis (5). Composed of minute convolutions of the epididymal duct, the epididymis appears solid due to its dense packing. The size of the duct gradually reduces in size as it travels from the head of the epididymis to the tail.

The ductus deferens commences at the tail of the epididymis, continuing the path of the epididymal duct. The sperms are stored and continue their maturation process within the ductus deferens. Also known as, vas deferens, it is the primary component of the spermatic cord and ends its course by joining the duct of the seminal gland to form the ejaculatory duct. Most of the veins from the ductus, including those from the distal pampiniform plexus, drain into the testicular vein. The final segment, however, drains into the vesicular/prostatic venous plexus (2, 5).

1.1.4. SEMINAL GLANDS AND EJACULATORY DUCTS

Each seminal gland, also known as a seminal vesicle, is an elongated structure situated between the base of the bladder and the rectum. Contrary to what the term "vesicle" might suggest, these glands do not serve as storage for sperm (2). Located obliquely above the prostate, their main function is to secrete a thick alkaline fluid containing fructose, providing energy for the sperm. Additionally, they produce a coagulating agent that combines with the sperm as they pass into the ejaculatory ducts and the urethra (6).

The ejaculatory ducts are narrow tubes that form through the conjunction of the seminal gland ducts and the ductus deferens. These ducts, approximately 2.5 cm in length, originate near the bladder's neck and run in proximity. They travel in an anterior-inferior direction

through the prostate's posterior section and along the sides of the prostatic utricle. They converge and open onto the seminal colliculus through tiny, slit-like openings located on or just within the prostatic utricle's entrance. Despite passing through the glandular part of the prostate, the prostate's secretions don't mix with the seminal fluid until after the ejaculatory ducts have ended in the prostatic urethra (2, 6).

1.1.5. PROSTATE GLAND

Roughly the size of a walnut, the prostate is the most prominent accessory gland of the male reproductive system. This firm gland surrounds the prostatic urethra. The glandular part constitutes approximately two-thirds of the prostate, while the remaining one-third is fibromuscular (2). The dense, neurovascular fibrous capsule of the prostate includes the prostatic plexuses of veins and nerves. All this is enclosed by the visceral layer of the pelvic fascia, which forms a fibrous sheath around the prostate. This sheath is thin in the front, blends laterally with the puboprostatic ligaments, and becomes thicker posteriorly where it merges with the rectovesical septum (7). The prostatic ducts, numbering between 20 and 30, primarily empty into the prostatic sinuses which are situated on both sides of the seminal colliculus on the posterior wall of the prostatic urethra.

The prostate secretes a thin, milky liquid that accounts for about 20% of the total volume of semen, which serves as the transportation medium for sperm. This prostatic fluid plays a critical role in activating the sperm. The arterial supply of the prostate is provided by the prostatic arteries. Conversely, the venous drainage from the prostate is carried out by veins joining to form a plexus around the prostate's sides and base, which subsequently drain into the internal iliac veins (2, 7).

1.2. PHYSIOLOGY OF THE MALE REPRODUCTIVE SYSTEM

The reproductive functions of the male system can be categorized into three main components. Firstly, the formation of sperm, also known as spermatogenesis. Secondly, the execution of the male sexual act, and thirdly, the regulation of male reproductive processes by a range of hormones. Alongside these reproductive functions, male sex hormones influence the accessory sexual organs, regulate cellular metabolism, control growth, and manage other functions of the body (8).

1.2.1 SPERMATOGENESIS

Spermatogenesis begins during the formation of the embryo when primordial germ cells move to the testes and turn into immature germ cells, known as spermatogonia, within the seminiferous tubules. These spermatogonia then travel among Sertoli cells towards the central lumen of the seminiferous tubules (9). At puberty, spermatogonia start mitotic division, continually multiplying and transform into primary spermatocytes, which undergo meiotic division, producing secondary spermatocytes. These secondary spermatocytes further divide to yield spermatids, which eventually mature into spermatozoa (8). Spermatogenesis occurs in response to stimulation from gonadotropic hormones of the anterior pituitary gland. A variety of hormones have a crucial role in this process, which we will explore in more detail later. Dysfunction in any of these stages can disrupt the whole process. Potentially, it can result in defective and/or diminished sperm production or in extreme cases, it could lead to a total absence of spermatozoa, causing infertility (10, 11).

1.2.2 HORMONES INVOLVED IN MALE REPRODUCTIVE FUNCTION

Several hormones are involved in the functioning of the male reproductive system, including follicle-stimulating hormones (FSH), luteinizing hormone (LH), testosterone, and inhibin B (8). However, it's the secretion of gonadotropin-releasing hormones (GnRH) from the hypothalamus that holds the major share of the control of sexual function. In response to GnRH, the anterior pituitary gland releases two gonadotropic hormones: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (12). In turn, LH promotes steroidogenesis by initiating a series of events at the mitochondrial level converting cholesterol into testosterone, and FSH primarily stimulates spermatogenesis by stimulating the Sertoli cells to ensure the conversion of the spermatids to sperm (10). GnRH is secreted periodically for a few minutes roughly every 1 to 3 hours. The anterior pituitary gland secretes LH in a rhythmic manner, closely following the episodic release of GnRH. In contrast, FSH secretion changes only slightly with each fluctuation of GnRH secretion and tends to adjust over an extended period, responding to more gradual shifts in GnRH levels (8).

LH and FSH are glycoproteins. They primarily impact their target tissues in the testes by activating the cyclic adenosine monophosphate second messenger system, which in turn trigger specific enzyme systems within the target cells. LH stimulates the secretion of testosterone by the interstitial cells of Leydig in the testes and the amount of LH available

correlates with the quantity of testosterone secreted. Testosterone exerts negative feedback on the hypothalamus in response to LH, leading to a reduced GnRH secretion, as shown in Figure 2. This reduction in GnRH results in decreased LH and FSH secretion, ultimately lowering testosterone levels due to diminished LH levels (12, 8).

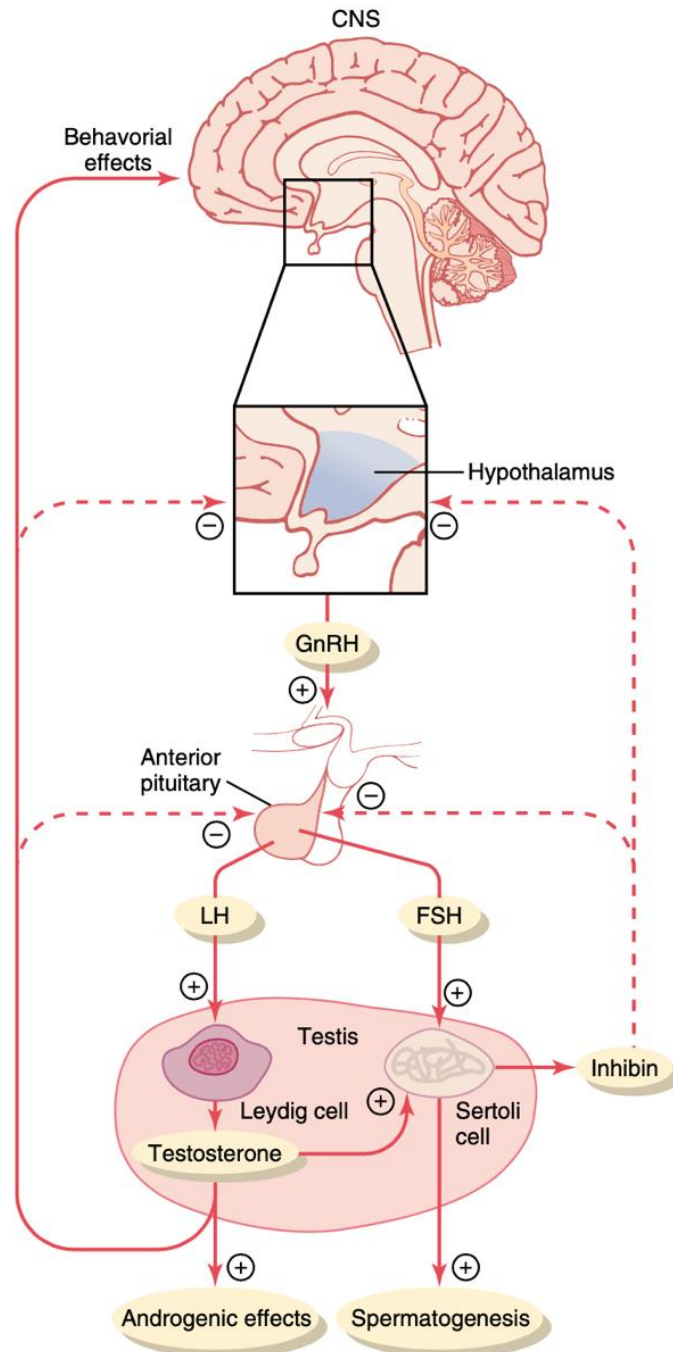


Figure 2. Feedback regulation of the hypothalamic-pituitary-testicular axis in males. Stimulatory effects are shown by + and negative feedback inhibitory effects are shown by - FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone (8)

To initiate spermatogenesis, both FSH and testosterone are required. FSH interacts with specific receptors on the Sertoli cells located within the seminiferous tubules, prompting these cells to proliferate and release essential substances for spermatogenesis. Simultaneously, testosterone which originates from the Leydig cells in the interstitial spaces diffuse into the seminiferous tubules, exerting a significant influence on the spermatogenic process (8).

If spermatogenesis occurs too quickly, the secretion of FSH from the pituitary gland diminishes. This feedback mechanism is thought to be due to the release of a hormone called inhibin B by the Sertoli cells (13). Inhibin B has a direct effect on the anterior pituitary gland, reducing FSH release and potentially having a minor effect on the hypothalamus, decreasing GnRH secretion. Like LH and FSH, inhibin B is a glycoprotein that has been isolated from cultured Sertoli cells. Its strong inhibitory effect on the anterior pituitary gland provides a crucial negative feedback mechanism for control of spermatogenesis. This operates concurrently and in line with the feedback system that regulates testosterone secretion (12, 8, 14).

1.3. VARICOCELE

Varicocele is a congenital condition characterized by an abnormal dilation of testicular veins in the pampiniform plexus in the spermatic cord (15, 16). It is frequently described as a “bag of worms” as a result of valvular incompetence within the testicular veins allowing transmission of hydrostatic venous pressure, as shown in Figure 3 (17). The left side is predominantly affected, 2% of cases are bilateral, and rarely isolated right-sided varicocele is found. In the latter case, prompt further investigation should be performed as it might indicate an abdominal or retroperitoneal mass (18).



Figure 3. Left varicocele in an adolescent boy (15)

1.1.1 ETIOLOGY

The development of primary varicocele, also called idiopathic varicocele, is believed to have multiple contributing factors. As already mentioned, the presence of incompetent venous valves leading to reflux and increased hydrostatic pressure occurring during puberty is a well-recognized theory in the field. As well, the anatomical variations in venous drainage between the left and right internal spermatic veins, which account for the increased prevalence of left-sided varicocele, form an integral part of this well-established theory (18). The exact pathophysiology of varicocele and its impact on male fertility potential remains unclear (19).

However, one plausible mechanism that is likely to play a role is scrotal hyperthermia, given its influence on endocrine function and spermatogenesis, both of which are known to be sensitive to elevated temperatures (18). Evidence suggests that smoking correlates with an increased risk of varicocele due to the accumulation of cadmium in the testes, which has the potential to induce testicular damage (20). Secondary varicoceles are the result of increased pressure due to a mass located in the retroperitoneal space, leading to an obstruction of venous drainage into the inferior vena cava or the left renal vein, or because of a thrombotic event (21).

1.1.2 EPIDEMIOLOGY

Varicocele is a common finding as it can affect up to 15% of the male population, accounting for 35% of cases of primary infertility and up to 80% of cases of secondary infertility. The occurrence of varicocele is observed in 5-15% of adolescents and rarely seen in the preadolescent age group (2-10 years) (15). A study was conducted to assess the prevalence of varicoceles among a cohort of 4052 boys aged 2-19. The results of the study demonstrated a prevalence of varicoceles of <1% among boys aged 2-10, 7.8% in boys aged 11-14, and 14.1% in boys aged 15-19. Based on these findings, it is highly suggested that venous incompetence occurs primarily during testicular development. It is associated with an increase in blood flow following the onset of puberty (18).

A different study suggested an age-related correlation in the prevalence of varicocele in adult men. These findings indicated that the occurrence of varicocele tends to increase by approximately 10% for each decade of life. The results showed the following distribution: 18% at age 30-39, 24% at age 40-49, 33% at age 50-59, 42% at age 60-69, 53% at age 70-79, and 75% at age 80-89 (18). Based on these epidemiological observations, it is indicated that testicular venous incompetence increases with age, which is likely attributed to the aging of the venous valves. These findings align with the increase in the prevalence of lower limb varicose veins observed with advancing age (18).

1.1.3 CLINICAL PICTURE

The typical symptom of varicocele appears as a painless para testicular mass. Occasionally, patients may experience persistent, mild pain in the affected testicle (15). They might describe it as a dragging-like, or aching pain, and a feeling of heaviness in the affected testicle. Rarely, the tortuous scrotum is apparent while the patient is in a supine position because it is decompressed, but by instructing the patient to stand and do the Valsalva maneuver the varicocele becomes more prominent. In the absence of symptoms, varicoceles may remain undetected until the investigation of male sub-fertility. Nowadays, most varicoceles are diagnosed incidentally during scrotal ultrasonography performed to evaluate testicular-related pain or other symptoms (22). Screening adolescents for varicocele is not routinely done by pediatricians (15).

1.1.4. CLINICAL DIAGNOSIS

According to the guidelines of the European Association of Urology (EAU) and the European Society for Pediatric Urology (ESPU), the initial diagnosis of varicocele should be made through a clinical examination while the patient is standing upright. As mentioned previously, the Valsalva maneuver can facilitate the diagnosis of varicocele as it allows for better visualization and palpation during the examination. A varicocele with a diameter exceeding 4 mm is typically noticeable in a clinical setting (22).

Following the guidelines established by Dubin and Amelar in 1970, varicocele is clinically diagnosed and classified as follows: Grade 1 is only palpable when the patient is performing a concurrent Valsalva maneuver; Grade 2 is palpable when the patient is standing, without performing a Valsalva maneuver; and Grade 3 is visible through the scrotal skin before performing palpation while the patient is standing (22, 15). Boys with a Grade 3 varicocele have a higher risk of experiencing a halt in testicular growth. The size of the testicles should be measured with the use of an orchidometer, or via scrotal sonography to detect testicular hypoplasia (15). Testis is considered hypoplastic if it is smaller by more than 2 ml or 20% compared to the other testis. If the affected testis, typically the left, is notably smaller than the other testis, it suggests that spermatogenesis has been negatively influenced. Sexually mature adolescents at Tanner stage V should be considered for semen analysis in such cases (16).

1.1.5. DIAGNOSTIC IMAGING

To confirm the diagnosis of varicocele the EAU recommends the use of ultrasonography with the patient both lying down and standing upright. The color Doppler is considered the gold-standard method. In addition to confirming the diagnosis, the use of color Doppler ultrasonography (CDUS) can provide additional information on the presence of venous reflux and the diameter of veins (21). With sensitivity and specificity close to 100%, CDUS displays high accuracy, as it also serves as a useful tool for staging varicocele according to the classification systems (15).

Multiple classification systems have been suggested, and among them, the ones developed by Sarteschi are the most widely used today. It is considered the most comprehensive classification due to its clearly defined examination technique and inclusion of most parameters assessed in various other classifications. The Sarteschi classification system is a method used to classify varicoceles into five grades based on various factors. These factors include the

presence of varicose veins when a person is either lying down or in a standing position, characteristics of the reflux, the size of the testis, and the relationship of the dilated veins to the testis (21, 23). Table 1 describes in further detail the Sarteschi classification.

Table 1. Ultrasonographic classifications of varicoceles. The Sarteschi classification (23).

Grades	
Grade 1	Inguinal reflux only during Valsalva in not enlarged vessels.
Grade 2	Supra-testicular varicosities with reflux only during Valsalva
Grade 3	Peri-testicular reflux only during Valsalva in enlarged vessels. Visible but not dilated vessels when supine. Enlarged when standing.
Grade 4	Enlarged vessels in supine and standing position, with increasing caliber during Valsalva. Reflux at rest, increasing during Valsalva. Possible testicular hypotrophy.
Grade 5	Enlarged vessels in supine and standing position, with caliber not increasing with Valsalva. Reflux at rest, not increasing during Valsalva. Testicular hypotrophy. Intratesticular varices may be present.

1.1.5. TREATMENT

In the treatment of varicoceles, the primary modality utilized today is varicocelectomy. The predominant objective is to enhance the fertility potential in patients (15). The American urological association / American Society for Reproductive Medicine (AUA/ASRM) guidelines recommend the subinguinal microsurgical technique which has shown the highest success as compared to other techniques. This approach is regarded as the gold standard as it is associated with lower risks of complications and recurrences (24). However, it is demanding technically making it not possible for all surgeons to use it and are encouraged to choose a technique aligning with their competence and comfort levels (16). Alternative techniques include ligation of the veins of the pampiniform plexus via laparoscopy or ligation of the internal spermatic vein in the retroperitoneum (15).

The ESPU recommends the following criteria for performing varicocelectomy in children and adolescents: (I) varicoceles associated with smaller testis size, (II) additional testicular conditions compromising fertility, (III) bilateral palpable varicoceles, (IV) compromised sperm quality in older adolescents, (V) symptomatic varicoceles. The guidelines highlight the lack of evidence supporting the early treatment of varicocele at pediatric age results in better andrological results compared to treatments performed later in life (25).

Furthermore, the ESPU guidelines emphasize that a reduction in the combined volume of both testes, when compared to standard measurements, can serve as a potential criterion for diagnosis, assuming standard measurements are available. The latter defines testicular hypoplasia as a condition where one testis is either diminished by more than 2 ml or has a size reduction of over 20% in comparison to its counterpart. Large varicocele resulting in physical or psychological distress should be considered for repair (16). The current guidelines do not suggest using hormonal levels as criteria for deciding whether to operate or not. Nevertheless, despite these guidelines, a clear consensus on the practical management of varicoceles, both for fertility and non-fertility concerns, remains elusive, and many areas of controversy remain (25, 26).

1.4. THE RELATIONSHIP BETWEEN VARICOCELE AND HORMONES

While the effects of varicocele on sperm production are established, the link between clinical varicocele and impaired hormonal production remains unclear (27). Several studies have explored the significance of monitoring hormonal levels when deciding on varicocele surgery. Hudson *et al.* conducted research investigating potential indicators, such as testicular size, varicocele grade, age, sperm density, duration of infertility, and certain hormonal levels, to predict improvements in seminal characteristics post-surgery. They concluded that these measures could potentially predict positive outcomes following VR in an infertile man with varicocele (28). Fideleff *et al.* researched the hormonal (FSH, LH, and testosterone) and histological characteristics of pediatric-adolescent varicocele to understand its natural progression and identify prognostic indicators of testicular damage. Their findings suggest that there isn't any reliable biochemical marker in children and adolescents that may predict impaired testicular function (29).

However, Guarino *et al.* investigated the relationship between testicular size, gonadotropin levels, and semen parameters in adolescents. They found a higher gonadotropin levels in patients with irregular semen parameters yet observed no correlation with testicular size measurements (30). The use of serum FSH as an indicator for spermatogenesis is conflicting. FSH levels appear to vary based on which stage of spermatogenesis is affected. For instance, FSH levels rise when azoospermia is due to compromised spermatogonia count and function. Whereas they remain within the normal range when the azoospermia results from a halt in maturation of the spermatocyte or spermatid level (12).

A widely accepted theory suggests that varicocele raises the scrotal internal temperature, resulting in reduced function of Sertoli cell and germinal cell damage. Sertoli cells are the principal site of inhibin B production which regulates FSH secretion by the pituitary gland. The increased scrotal temperature due to varicocele compromises spermatid's function, leading to reduced production of inhibin B. Research on adult varicocele patients found a positive correlation between serum inhibin b levels and sperm concentration following VR (14). Pierik *et al.* observed a rise in inhibin B levels after varicocele surgery, indicating that such treatment might facilitate the functional recovery of Sertoli cells, positively impacting fertility. These authors also noted a correlation between elevated levels of inhibin B and an increase in sperm count/concentration, as well as improved motility (31).

While Romeo *et al.* found that inhibin B levels are decreased in adolescents with untreated varicocele and directly relate to testicular volume. They concluded that this might serve as an early indicator of Sertoli cell damage, potentially affecting spermatogenesis. It could also represent a new indication for varicocele repair (32). Another study revealed that adolescents with idiopathic varicocele have lower inhibin B levels compared to healthy controls. After surgical intervention for varicocele, there's an observed increase in inhibin B concentration, supporting the significance of early treatment to ensure fast spermatogenesis recovery. The level of inhibin B proved to be a valid marker for assessing varicocele's impact on testicular functionality (14). However, conducting randomized controlled studies is essential to better clarify the hormonal attributes of adolescents with varicocele (12).

2. OBJECTIVES

2.1 AIM OF THE STUDY

The purpose of the study is to examine the hormonal levels of adolescents with varicocele, specifically focusing on FSH, LH, and inhibin B, treated and followed at Pediatric Surgery University Hospital of Split. We aim to determine the prevalence of normal, high, or low levels of these hormones in the patient cohort and assess if routine hormonal tests offer any significant diagnostic or prognostic value in the management of varicocele.

2.2 HYPOTHESIS

1. The hormonal levels of LH and/or FSH of most patients with varicocele monitored at University Hospital of Split are within the normal range before surgery.
2. There is no difference in hormone levels of LH and/or FSH in the operated group compared to the non-operated group.
3. The hormonal level of inhibin B of most patients with varicocele monitored at University Hospital of Split are within the normal range before surgery.
4. The hormone level of inhibin B of the operated patient group will be in the lower range of the normal level of inhibin B compared to the non-operated patient group.

3. PATIENTS AND METHODS

3.1 STUDY DESIGN

This study was designed as a retrospective cohort study conducted at the Department of Pediatric Surgery, University Hospital of Split in Croatia. All data was collected from the hospital's patient records between 1st May 2015 to 1st June 2023.

3.2 DATA COLLECTION

This cohort study included a total of 120 participants, all patients were between the age of 11 to 21, with the median age being 16. While the pediatric population is up to 18 years of age, the study included patients who began their monitoring at the Split Pediatric Surgery Clinic before they turned 18 and continued to be monitored at the Clinic past that age. The data collection was exclusively extracted from the Department of Pediatric Surgery at the University hospital of Split. The health records were gathered by either my mentor or me. Spanning from 2015 to 2021, included exclusively patients treated by a single surgeon. This dataset both included operated and non-operated patients. Exclusion criteria for this period involved the absence of recorded LH, FSH, or Inhibin B hormonal levels. For the period 2021 to 2023, the data was collected from the hospital's system, including patients treated by other surgeons as well. The same exclusion criteria were applied to ensure that only records with complete hormonal profiles were used in this study. We recorded the patient age, FSH, LH and inhibin B hormonal levels, and whether the patient had undergone surgery. This data was then organized into tables using Microsoft Excel. The final dataset is anonymized, ensuring no personal patient details are revealed.

3.3 STATISTICAL ANALYSIS

All statistical calculations were performed using IBM SPSS version 27.0.1. The analysis involved both descriptive and inferential statistical tests. Descriptive statistics were used to summarize and describe the characteristics of the study participants like, age. Frequencies and percentages were calculated for categorical variables like levels of hormone FSH, LH and Inhibin B. For continuous variables like values of FSH, LH, Inhibin B mean, and standard deviation was calculated. Data distribution was estimated using Kolmogorov-Smirnov and Shapiro-Wilk test. Mann-Whitney U Test was conducted to find difference in FSH, LH and Inhibin B values between operated and non-operated patients for non-normal distribution of

data. T-test was used for normal distribution of data. The significance level for all statistical tests was set at $p < 0.05$, indicating a 95% Confidence Interval.

3.4 COMPLIANCE WITH ETHICAL STANDARDS

All data and rights of patients were protected in accordance with the 1964 Helsinki declaration, including its subsequent updates or equivalent ethical norms. The study was ethically approved by the Ethics Review Board of University Hospital of Split under reference No. 2181-147/01/06/LJ.Z.-23-02 on 21st July 2023.

4. RESULTS

This study included a total of 120 patients diagnosed with varicocele. The age of these patients ranged between 15.34 to 16.35 years, as indicated by the confidence interval. With a median age of 16.00, the age distribution appears potentially symmetrical. While the ages extended up to 21 years for some participants, the majority were concentrated around the median. The standard deviation and variance provide insights into the degree of spread or dispersion of ages around the mean. The interquartile range gives an idea about the middle 50% of the age distribution (Table 1).

Table 1. Age description

Parameters		Statistic
Age	Mean	15.84
	95% Confidence Interval for Mean	
	Lower Bound	15.34
	Upper Bound	16.35
	5% Trimmed Mean	15.88
	Median	16.00
	Variance	4.039
	Std. Deviation	2.010
	Minimum	11
	Maximum	21
	Range	10
	Interquartile Range	3

Table 2 presents mean hormone values in patients. For Follicle-Stimulating Hormone (FSH), the mean is 4.2 (SD = 3), with a range from 1 to 18. Luteinizing Hormone (LH) has a mean of 3.8 (SD = 2), ranging from 1 to 11. Inhibin B shows a mean of 170 (SD = 80), with values between 18 and 324. These results provide insights into hormone levels and variability among patients.

Table 2. Mean values of hormones in patients

	Mean	Maximum	Minimum	Standard Deviation
FSH	4.2	18	1	3
LH	3.8	11	1	2
Inhibin B	170	324	18	80

Note. FSH – Follicle-stimulating hormones, LH – Luteinizing hormone

Majority of participants have FSH levels within the normal reference range of 1.5 to 12.9. However, there are a small number of participants with low FSH levels (4.2% of the total) and an even smaller number with high FSH levels (1.7% of the total). Majority of participants have LH levels within the normal reference range of 1.3 to 9.8. However, a small number of participants have LH levels that are either high (0.8% of the total) or low (2.5% of the total). There are 2 participants (4.0% of the total) with low Inhibin B levels. Most participants (46 participants, 96.0% of the total) have Inhibin B levels within the normal range as shown in Table 3.

Table 3. Levels of hormones in patients.

	High		Low		Normal	
	Count	%	Count	%	Count	%
FSH	2	1.7%	5	4.2%	110	94.7%
LH	1	0.8%	3	2.5%	114	97%
Inhibin B	2	4%			46	96%

Note. FSH – Follicle-stimulating hormones, LH – Luteinizing hormone

A total of 75% of patients has not undergone the operation while 25% of them have undergone the operation shown in Table 4.

Table 4. Percentage of operated patients vs non-operated

	Count	%
Not operated	90	75.0%
Operated	30	25.0%

Table 5 shows that the Independent-Samples t-Test was utilized to compare the distributions of LH, Inhibin B levels between the operated and non-operated groups. The test statistics shows that there is no significant difference in the levels of LH; ($p = 0.081$) between operated and non-operated patients. However, there is statistically significant evidence to conclude that the Inhibin B levels; ($p = 0.008$) were higher for the non-operated ($M=187$, $SD=77$) than operated ($M=119$, $SD=66$) patients. Mann-Whitney U Test was used to compare the distribution of FSH values between operated and non-operated patients. There was no significant difference in the FSH values for operated ($M=5$, $SD=4$) and non-operated patients ($M=3.7$, $SD=2$) conditions; ($p = 0.075$)

Table 5. Hormone values in operated and non-operated

	FSH		LH		Inhibin B	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Not operated	3.7	2	3.7	2	187	77
Operated	5	4	4.5	3	119.6	66
<i>P</i>	0.075		0.081		0.008	

Note. FSH – Follicle-stimulating hormones, LH – Luteinizing hormone

The power analysis indicated that the test for mean difference has a power of approximately 0.570. This value represents the probability of correctly rejecting the null hypothesis when there is indeed a true mean difference between two groups. Additionally statistical test has a 57% chance of detecting this effect and producing a statistically significant result.

Table 6. Power analysis table

	Power	Test Assumptions					P
		N1	Std. Dev1	N2	Std. Dev2	Mean Difference	
Test	0.570	30	4.02	90	1.82	1.670	0.05

Note. Std - Standard

For Follicle-Stimulating Hormone (FSH), the Kolmogorov-Smirnov test yielded a statistic of 0.205 ($p < 0.001$), and the Shapiro-Wilk test produced a statistic of 0.811 ($p < 0.001$), indicating non-normality of the FSH data. Regarding Luteinizing Hormone (LH), the Kolmogorov-Smirnov test statistic was 0.091 ($p = 0.200^*$), while the Shapiro-Wilk test yielded a statistic of 0.958 ($p = 0.105$), suggesting potential normality. Similarly, for Inhibin B, the Kolmogorov-Smirnov test produced a statistic of 0.101 ($p = 0.200^*$), and the Shapiro-Wilk test yielded a statistic of 0.971 ($p = 0.317$), indicating that the assumption of normality is firmly established.

Table 7. Tests of normality

	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
FSH	0.205	45	0.000	0.811	45	0.000
LH	0.091	45	0.200*	0.958	45	0.105
Inhibin B	0.101	45	0.200*	0.971	45	0.317

Note. FSH – Follicle-stimulating hormones, LH – Luteinizing hormone df – degrees of freedom, Sig – significance for the test

5. DISCUSSION

Varicocele, a dilation of the pampiniform venous plexus of the spermatic cord, has been of particular interest in the field of andrology and pediatric surgery due to its possible impact on male infertility (27). The primary objective of varicocele therapy is to maintain or re-establish fertility.

Yet, for adolescents with varicocele, there is controversy over which clinical parameters or findings might predict eventual infertility. The “Report on varicocele and infertility” from the ASRM Practice Committee, provide the following indication, adolescent males with varicoceles and signs of reduced testicular size on the affected side could be candidates for varicocele repair. If there’s no clear evidence of testicle size reduction, these adolescents should have yearly measurements of testicle size and/or semen test to spot any indications of varicocele-caused testicular damage (33). Early identification and treatment during adolescence can potentially reduce these risks, even though, as stated by the ASMR Practice Committee, “*data are lacking regarding the impact on future fertility*” (27). Which then make sense that carrying out prophylactic surgery on all adolescents diagnosed with varicocele might lead to many unnecessary procedures (27).

For now, semen analysis is perhaps the most reliable indicator of fertility in adolescent boys without trying for paternity, but even abnormal results don’t always predict future fertility problems. While the WHO provides reference ranges for semen parameters, the ideal values for adolescents remain unclear (34). Chu *et al.* conducted a study on Tanner V boys and found that up to 47% of these patients with initially abnormal SA, recovered naturally without surgery.

However, a minor group consistently showed poor total mobile count (TMC), indicating the need for future focused research and intervention (27). Even if semen analysis has a critical role in the follow up of post-pubertal boys with varicocele, they aren’t always performed. Obtaining an SA might be challenging due to personal, familial, religious beliefs, or even challenges from the physician’s side (35). Additionally, getting an SA is time demanding, requires specific facilities, and possible cost for the patient’s family. Therefore, alternative indicators for abnormal semen might be necessary for those that are unable or incapable of providing samples (27).

This cohort study carried out at the Pediatric Surgery Department of Split Hospital, was conducted to assess whether the hormonal levels of FSH, LH and inhibin B were within the normal range prior to surgery, and if there was any correlation between these levels in patients who got operated and those who did not. The aim of this study was to evaluate the importance of routinely performing hormonal test on each new patient and maintaining these tests during subsequent follow-ups, considering the uncertainty surrounding this topic. From our sample of

120 participants, a significant majority exhibited hormonal levels of FSH, LH and inhibin B within the normal range before any surgery. Specifically, only 5.9% had abnormal FSH values based on the hospital's standard range (1.5-12.9 mIU/mL). Additionally, 3.3% presented with LH levels outside the 1.3 to 9.8 mIU/mL interval, while 4% had a level of inhibin B outside the range of 25-325 mIU/mL. These findings correlate with the observations of Romeo *et al.*, who reported no significant differences in basal or GnRH-stimulated FSH, LH, or basal testosterone between pubertal boys (Tanner 4 and 5) with varicoceles and the age-matched group (32). In contrast, Romeo *et al.* did report a decrease in inhibin B levels among the varicocele group (32).

Our study on the other hand, identified a statistically significant difference in inhibin B levels between the operated and non-operated group. Those who underwent surgery were more likely to have inhibin B values in the lower half of the spectrum compared to the non-operated group. This observation aligns with Molinaro *et al.*, who documented that adolescents with idiopathic varicocele typically have diminished inhibin B levels, which tend to rise postoperatively (14). Nevertheless, no statistically significant difference was found between operated and non-operated participants concerning FSH and LH levels, a finding consistent with the work of Fideleff *et al.* (29). The results in this study suggest that consistent testing for FSH and LH may not significantly influence the surgical decision-making process. Supported by previous studies, these hormones may not be the best predictors of future infertility and adopting a more selective approach could be more beneficial (29, 32).

In contrast, our study indicates that inhibin B levels could be a potential early marker of Sertoli cell damage and could thereby serve as a new indicator for recommending varicocele repair. However, further research is necessarily for more definitive conclusions.

Moreover, it's crucial to acknowledge the limitations of this study. Firstly, our sample size, the study included a relatively small sample size of 120 patients, which may limit the generalizability of the findings to a broader population of individuals with varicocele. Secondly, most of the patients were treated by a single surgeon, which could further limit the findings. Incomplete data collection can lead to missing data, potentially introducing bias, and affecting the validity of the study's conclusions. It is known that hormonal fluctuations can be influenced by various factors and the study did not address potential confounding variables that might affect hormone measurements. The hormone levels were taken at a single point in time, which may not capture the full variability in hormone levels over time. Furthermore, a control group would have helped to differentiate between varicocele-related effects and normal hormonal variations. The study used specific reference ranges for hormone levels (e.g., normal FSH range of 1.5 to 12.9 mIU/mL). These reference ranges may vary across different laboratories,

potentially affecting the interpretation of hormone levels. Lastly, conducting the study at a single center may limit the diversity of the patient population and the generalizability of the results to different geographic locations, ethnicities, and socioeconomic backgrounds.

6. CONCLUSION

In conclusion, this study revealed no statistically significant difference between FSH and LH values in operated and non-operated adolescents. Majority of the participants had hormone values within the normal range, with 94.7% for FSH and 97% for LH. However, a statistically significant difference was found between the inhibin B values in operated and non-operated. In both non-operated and operated patients, inhibin B levels remained within the normal range. However, those who underwent surgery were more likely to have inhibin B values in the lower half of the spectrum compared to their non-operated counterparts. The relevance of monitoring FSH and LH levels does not appear to influence the decision to proceed with varicocelelectomy. Further investigations should be done to determine the importance of inhibin B levels in guiding the decision to undergo varicocelelectomy.

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8. SUMMARY

Objectives: To examine the hormonal levels of adolescents with varicocele, specifically focusing on FSH, LH, and inhibin B, treated and followed at Department of Pediatric Surgery at University Hospital of Split. We aim to determine the prevalence of normal, high, or low levels of these hormones in the patient cohort and assess if routine hormonal tests offer any significant diagnostic or prognostic value in the management of varicocele.

Materials and methods: This study was designed as a retrospective cohort study conducted at the Department of Pediatric Surgery, University Hospital of Split in Croatia. All data was collected from the hospital's patient records between 1st of May 2015 to 1st June 2023. This cohort study included a total of 120 participants. The data collection was exclusively extracted from the Department of Pediatric Surgery at the University hospital of Split. Spanning from 2015 to 2021, included exclusively patients treated by a single surgeon. Exclusion criteria for this period involved the absence of recorded LH, FSH, or Inhibin B hormonal levels. For the period 2021 to 2023, the data was collected from the hospital's system, including patients treated by other surgeons as well. We recorded the patient age, FSH, LH and inhibin B hormonal levels, and whether the patient had undergone surgery.

Results: Majority of participants have FSH, LH and Inhibin B levels within the normal reference range. The test statistics shows that there is no significant difference in the levels of LH;(p = 0.081) between operated and non-operated patients. However, there is statistically significant evidence to conclude that the Inhibin B levels;(p = 0.008) were higher for the non-operated (M=187, SD=77) than operated (M=119, SD=66) patients. There was no significant difference in the FSH values for operated (M=5, SD=4) and non-operated patients (M=3.7, SD=2) conditions; (p = 0.075).

Conclusions: In conclusion, this study revealed no statistically significant difference between FSH and LH values in operated and non-operated adolescents. However, a statistically significant difference was found between the inhibin B values in operated and non-operated. However, those who underwent surgery were more likely to have inhibin B values in the lower half of the spectrum compared to their non-operated counterparts. The relevance of monitoring FSH and LH levels does not appear to influence the decision to proceed with varicocelectomy. Further investigations should be done to determine the importance of inhibin B levels in guiding the decision to undergo varicocelectomy.

9. CROATIAN SUMMARY

Cilj: Ispitati razine hormona u adolescenata s varikokelom, s posebnim naglaskom na FSH, LH i inhibin B, liječenih i praćenih u Klinici za dječju kirurgiju Kliničkog Bolničkog Centra (KBC) Split. Cilj nam je bio u skupini ispitanika odrediti prevalenciju normalnih, visokih ili niskih razina ovih hormona i procijeniti imaju li rutinski hormonski testovi značajnu dijagnostičku ili prognostičku vrijednost u liječenju varikokele.

Materiali i metode: Ovaj rad je osmišljen kao retrospektivno kohortno ispitivanje provedeno u Klinici za dječju kirurgiju KBC-a Split, Podatci su prikupljeni iz medicinske dokumentacije bolesnika liječenih u razdoblju od 1. svibnja 2015. do 1. siječnja 2023. Ispitivanjem je obuhvaćeno 120 ispitanika. Podatci prikupljeni u razdoblju od 2015. do 2021. odnose se na bolesnike liječene od strane jednog liječnika. U istraživanje su uključeni samo oni bolesnici s nalazima LH, FSH i inhibina B. U razdoblju od 2021-2023 podatci su prikupljeni iz bolničkog informacijskog sustava te su uključeni i bolesnici drugih liječnika s istim kriterijima. Prikupljeni su podaci o dobi bolesnika, razinama hormona FSH, LH i inhibina B te je li bolesnik podvrgnut operaciji ili ne.

Resultati: Većina ispitanika je imala razine FSH, LH i inhibina B unutar referentnih vrijednosti. Statistička obrada nije pokazala statistički značajnu razliku u razinama LH ($p = 0.081$) između operiranih i neoperiranih bolesnika. Postoji statistička značajnost za zaključak da su razine inhibina B; ($p = 0.008$) bile više u neoperiranih ($M=187, SD=77$) od operiranih bolesnika ($M=187, SD=77$). Nije bilo statističke razlike u vrijednostima FSH; ($p = 0.075$) operiranih ($M=5, SD=4$) i neoperiranih bolesnika ($M=3.7, SD=2$).

Zaključak: Istraživanje nije pokazalo sttistički značajne razlike u vrijednostima FSH i LH između operiranih i neoperiranih adolescenata. Postoji sttistički značajna razlika u vrijednostima inhibina B operiranih i neoperiranih bolesnika. Bolesnici koji su podvrgnuti operaciji će vjerojatnije imati vrijednosti inhibina B u donjem dijelu normalnog raspona vrijednosti od skupine neoperiranih bolesnika. Praćenje razina FSH i LH se nije pokazalo relevantnim u donošenju odluke o potrebi varikokelektomije. Potrebno je provesti dodatna istraživanja kako bi se utvrdila važnost razina inhibina B u odlučivanju potrebe za varikokelektomijom.