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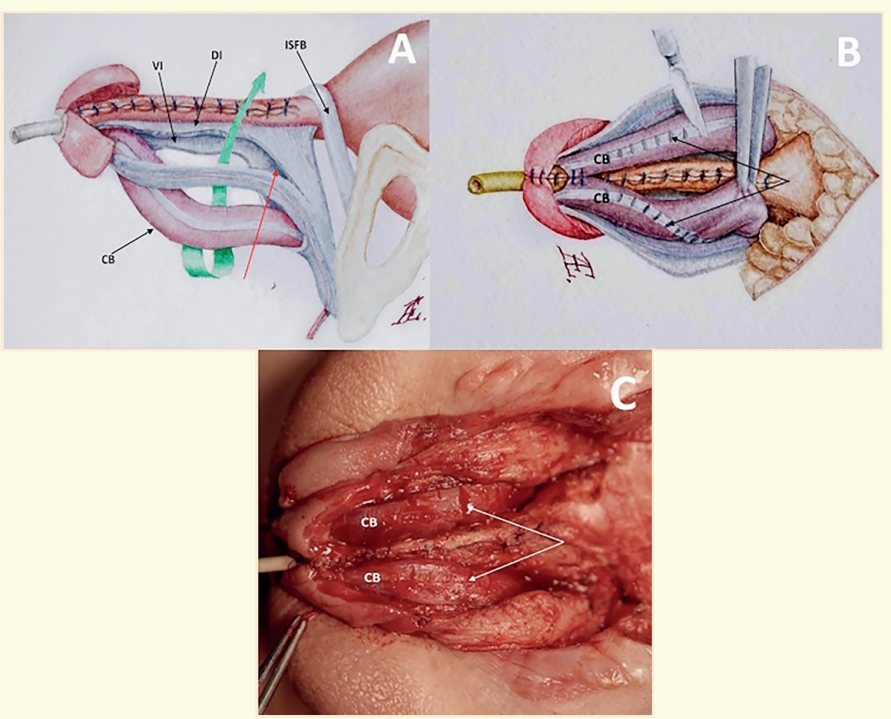

Cavernous body relocation. (Page 588)

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Correspondence and Editorial Address:

Rua Real Grandeza, 108 - conj. 101 - 22281-034 — Rio de Janeiro — RJ — Brazil
Tel.: + 55 21 2246-4003; E-mail: brazjurol@brazjurol.com.br

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doi: 10.1590/S1677-5538.IBJU.2024.05.01

Risk of infection during percutaneous nephrolithotomy is the hot topic in this number of the International Brazilian Journal of Urology

Luciano A. Favorito [1, 2]

[1] *Unidade de Pesquisa Urogenital - Universidade do Estado do Rio de Janeiro - Uerj, Rio de Janeiro, RJ, Brasil;* [2] *Serviço de Urologia, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brasil*

The September-October number of I*nt Braz J Urol* is the 30nd under my supervision. In this number the Int Braz J Urol presents original contributions with a lot of interesting papers in different fields: Robotic Surgery, BPH, Varicocele, Testicular migration, Prostate Cancer, Prostate Biopsy, Bladder Extrophy, Endourology and Infertility. The papers came from many different countries such as Brazil, Canada, Spain, Russia, USA, Germany and China, and as usual the editor´s comment highlights some of them. The editor in chief would like to highlight the following works:

Dr. Danilovic and collegues from Brazil, presented in page 561 (1) a nice study about the high-risk patients for septic shock after percutaneous nephrolithotomy, a recurrent topic in recent years in this Journal (2-4). The authors identified risk factors for urinary septic shock in patients who underwent percutaneous nephrolithotomy (PCNL) and concluded that patients with larger stones, positive preoperative urine culture, and a higher CCI are at risk for urinary septic shock after PCNL. These findings are of utmost importance for optimizing the perioperative care of these patients to prevent life-threatening complications.

Dr. Neto and collegues from Brazil, presented in page 530 (5) a important review about the addressing Oxidative Stress and Sperm DNA fragmentation in Varicocele-Affected Subfertile Men and concluded that the impact of varicocele grade and laterality on oxidative stress (OS) and sperm DNA fragmentation, as well as the effect of improved OS and sperm DNA fragmentation levels in pregnancy and live birth rates after varicocelectomy, are still unclear and deserve further investigation.

Dr. Favorito and Collegues from Urogential Reseacrh Unit - Brazil performed in page 519 (6) a nice review about the role of gubernaculum testis inervation in testicular migration and concluded that gubernaculum testis has important structural alterations during the testicular migration and the genitofemoral nerve and CGRP gene are of great importance in this process. The genitofemoral nerve provides motor innervation to the cremaster muscle and gubernaculum, which helps regulate the position of the testes within the scrotum.

Drs. Nikolaev and Demin from Russia performed in page 585 the cover paper of this edition (7). The authors proposed that the mobilization of the corpora under Buck's fascia, their dorsal translocation through the incisions in Buck's fascia and suturing corporal convex sides above the urethra would allow extend corporal covering of the urethra, reducing the risk of urethra-cutaneous fistula formation and concluded that the modified technique of incomplete penile disassembly applied in a homogenous group of patients with classic bladder exstrophy allows penile

shaft elongation, im- proved aesthetic outcomes, preserved erections, and eliminates dorsal curvature. The technique demonstrated feasibility and reliability while maintaining positive effects on tissue circulation. The absence of urethra-cutaneous fistulae is attributed to the complete corporal covering of the urethral sutures and supports the initial hypothesis.

Dr. Morote and Collegues form Spain performed on page 595 (8) a nice study about validation of the Barcelona-MRI predictive model (BCN-MRI PM) when PI-RADS v2.1 is used with transperineal prostate biopsies and concluded that the BCN-MRI PM has been successfully validated when mpMRI was reported with the PI-RADS v2.1 and prostate biopsies were conducted via the transrectal and transperineal route.

Dr. Augustyniak and collegues from Canada, Italy, Brazil and Germany performed on page 631 (9) a nice study about the challenges, barriers and educational gaps of physicians and laboratory specialists involved in human fertility care during the COVID-19 pandemic and concluded that there is an additional need to better understand the required changes in policies and organizational processes that would facilitate access to andrology services for male infertility and specialized care, as needed.

The Editor-in-chief expects everyone to enjoy reading.

CONFLICT OF INTEREST

None declared.

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Luciano A. Favorito, MD, PhD
Unidade de Pesquisa Urogenital
da Universidade do Estado de Rio de Janeiro - UERJ,
Rio de Janeiro, RJ, Brasil
E-mail: lufavorito@yahoo.com.br

ARTICLE INFO

Luciano A. Favorito
https://orcid.org/0000-0003-1562-6068

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REVIEW ARTICLE

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Role of Gubernaculum testis inervation during the process of testicular migration in human fetuses

Luciano A. Favorito [1], Laura M. M. Favorito [1], Ana R. M. Morais [1], Francisco J. B. Sampaio [1]

[1] *Unidade de Pesquisa Urogenital, Departamento de Anatomia da Universidade do Estado do Rio de Janeiro – UERJ, Rio de Janeiro, RJ, Brasil*

ABSTRACT

Purpose: The gubernaculum seems to be the most important anatomical structure in the testicular migration process. The objective of this paper is to review current literature regarding the role of gubernaculum testis nerves in testicular migration. We conducted a comprehensive literature review about the gubernaculum testis innervation. A PubMed database search was performed in April 2024, focusing on gubernaculum testis and cryptorchidism and genitofemoral nerve (GFN) and calcitonin gene-related peptide (CGRP) gene. The gubernaculum has its own nerve supply, the GFN, descending on the anteromedial surface of the psoas muscle from L1-L2 segments. The second phase of testicular descent is regulated by androgens and CGRP, released from the sensory nucleus of the GFN. The GFN doesn't directly play a role in testicular migration but there is a theory that shows a regulatory function of this nerve in hormonal action during this process. The gubernaculum testis has important structural alterations during the testicular migration and the genitofemoral nerve and CGRP gene are of great importance in this process. The genitofemoral nerve provides motor innervation to the cremaster muscle and gubernaculum, which helps regulate the position of the testes within the scrotum.

ARTICLE INFO

Luciano Alves favorito
https://orcid.org/0000-0003-1562-6068

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INTRODUCTION

During the human fetal period the testes migrate from the abdomen to the scrotum traversing the abdominal wall and the inguinal canal between the 15th and the 28th week post-conception (WPC) (1, 2). The most important factors involved in this process are: (1) intra-abdominal pressure (3); (b) development of the epididymis, spermatic vases and deferent ducts (4); (c) the genitofemoral nerve stimulus (5); (d) the hormonal stimulus (6-8); and (e) the gubernaculums development (9, 10).

The gubernaculum seems to be the most important anatomical structure in the testicular migration process, by means of contraction and shortening, thus imposing traction strength on the testis and facilitates the transition of the testis through the inguinal canal (9, 11, 12).

One of the factors involved in cryptorchidism is the failure of the gubernaculum to migrate all the way to the scrotum (13). Structural studies conducted in patients with cryptorchidism reveal significant changes in the gubernaculum's structure, with a higher quantity of fibrous tissue and lower concentration of collagen than in the fetal gubernaculum (14). Studies about the gubernaculum nerves and their role in testicular migration are scarce in literature. The objective of this paper is to review the current literature regarding the role of gubernaculum testis nerves in testicular migration.

MATERIAL AND METHODS

In this study we carried out a review about the role of the innervation of the gubernaculum testis in testicular migration and analyzed papers published in the past 50 years. A PubMed database search was conducted in April 2024 using the following Medical Subject Heading (MeSH) terms: "Testicular Migration" or 'Gubernaculum Testis' and either 'Genitofemoral Nerve" or "CGRP gene" or 'Undescended testis' or 'Cryptorchidism. Multiple free text searches were performed using the following terms individually through all fields of the records: 'Genitofemoral Nerve" or "CGRP gene", 'Undescended Testis', and 'gubernaculum testis' or "Testicular Migration". In this review we found several papers in these databases, and we included only papers in English and excluded case reports, editorials and opinions of specialists.

RESULTS

Testicular descent is regulated by hormonal and mechanic factors such the testosterone, insulin-like factor 3 (INSL3) and the gubernaculum (1, 15). The gubernaculum is a ligament-like structure that guides the testes into the scrotum during fetal development (16-18). The process of testicular migration is very complex process and occurs in two distinct phases: Phase 1: Abdominal stage and Phase 2: Inguinal-scrotal stage (1, 15-19). We show in this review some important aspects about the stages of testicular migration, the gubernaculum testis structure and innervation and the role of the genitofemoral nerve in this process.

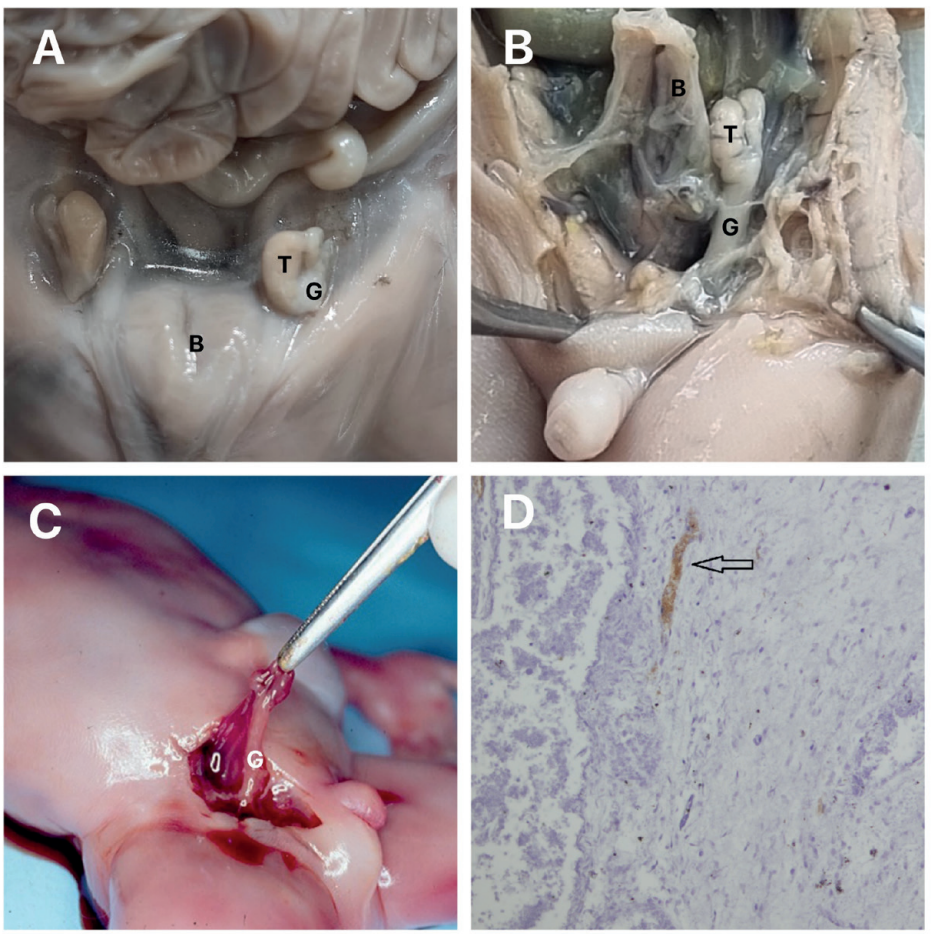
Abdominal Stage of testicular Migration

In this stage that begins around the 8th WPC and lasts until the 15th WPC the testis migrates from the abdomen to the internal inguinal ring when insulin-like hormone 3 (Insl3) from the Leydig cells stimulates the gubernaculum to swell, thereby anchoring the testis near the future inguinal canal as the fetus grows (Figure-1) (20).

During the eighth week of gestation, the testis and mesonephros are linked to the posterior abdomen wall by a peritoneal fold (21, 22). The portion of this fold called the diaphragmatic ligament degenerates, turning into the cranial portion of the gonadal mesentery. This structure is called the caudal gonadal ligament, which gives rise to the gubernaculum testis (1, 22). One of the factors involved in cryptorchidism is the failure of the gubernaculum to migrate all the way to the scrotum (1, 12, 22). The influence of fetal androgens on the fetal gubernaculum's development is very important for the alterations of this structure, and the changes in its secretions can be one of the factors involved in cryptorchidism (1).

In the abdominal stage of testicular migration, the gubernaculum enlarges to hold the testes near the

Figure 1 - Gubernaculum Testis.



The figure shows some aspects of the gubernaculum testis during testicular migration. A) A fetus with 20 weeks post conception (WPC), the abdominal wall was opened and we can observe the testis in abdominal position (T) and the gubernaculum (G) near to internal inguinal ring, B= Bladder; B) In this figure we can observe a fetuses with 21WPC, the inguinal canal was opened to show the relationship of gubernaculum testis(G) with the inguinal canal, T=Testis, B=Bladder; C) The figure shows a fetuses with 24WPC with the testis in inguinal position, we can observe the dissection of the gubernaculum testis (G) and D) In this picture we can observe a photomicrography of the gubernaculum testis in a fetus with 20WPC in abdominal position. The arrow shows the gubernaculum innervation, Immunohistochemycal with tubuline antibody X400.

groin, regulated by INLS-3 (23). INSL-3 is secreted by the Leydig cells and controls gubernaculum swelling via its receptor, LGR8 (leucine-rich repeat containing G protein-coupled receptor 8, also known as GREAT or relaxin receptor 2), a process resulting in thickening of the gubernaculum because of increases in water, glycosaminoglycan and hyaluronic acid content (1, 2, 23). At this moment, the future inguinal canal is still only a space in the musculature of the anterior abdominal wall, where only mesenchyme tissue exists. In this region, the genital branch of the genitofemoral nerve crosses the abdominal wall and descends to the scrotum where it will innervate the cremaster muscle, and subsequently, in the caudal to cranial direction, will provide the nerve supply to the gubernaculum (1, 19).

Inguinal-Scrotal Stage of testicular Migration

The inguinal-scrotal stage is the transition of the testes through the inguinal canal until their definitive arrival in the scrotum that begins around the 20th WPC and lasts until the 30th WPC (1, 23). During this stage, after the testis crosses the external inguinal ring the gubernaculum migrates across the pubic region to

reach the scrotum under control of testosterone. The androgen acts indirectly via the GFN, which produces CGRP to control the direction of migration (24).

The passage of the testis through the inguinal canal occurs very quickly between 21 and 25 WPC (1-4, 25). In a recent paper with more than 240 human male fetuses studied shows that all the fetuses older than 30 weeks already had the testes in the scrotum (25). Other authors, however, report that the testicular migration is only completed after the 32nd WPC (1). Heyns (1) found only 2.6% of the testes examined in his sample located in the inguinal canal, while Sampaio & Favorito (2), in a sample of 71 human fetuses, found 20.5% of the testes located there. Furthermore, 73.3% of these testes were in fetuses with ages between 21 and 25 WPC, indicating that in this period the migration through the inguinal canal intensifies. In the same study, all the fetuses older than 30 weeks already had the testes in the scrotum. Other authors, however, report that the testicular migration is only completed after the 32nd week post-conception (19).

The androgens stimulate growth and differentiation of the muscular part of the gubernaculum bulb, which facilitates the movement of the gubernaculum through the inguinal region by the traction resulting from this growth (12, 26). Distally the gubernaculum approaches the inguinal region. At this moment, the future inguinal canal is still only a space in the musculature of the anterior abdominal wall, where only mesenchyme tissue exists. In this region, the genital branch of the GFN crosses the abdominal wall and descends to the scrotum where it will innervate the cremaster muscle, and subsequently, in the caudal to cranial direction, will provide the nerve supply to the gubernaculum (1, 27, 28).

Gubernaculum Testis

The gubernaculum starts to develop in the human fetus during the sixth week of gestation, the same period when the germinative cells are arriving at the genital ridge (1, 19).

In the eighth week of gestation, the testis and mesonephros are linked to the posterior abdomen wall by a peritoneal fold. As the mesonephros degenerates, the portion of this fold cranial to the testis, called the diaphragmatic ligament, also degenerates, turning into the cranial portion of the gonadal mesentery. This structure is called the caudal gonadal ligament, which gives rise to the gubernaculum testis (1, 19).

At about the eighth week of gestation, a portion of the epithelium starts a small invagination from the coelomic cavity, across from the gubernaculum, slowly penetrating its mesenchymal substance. This invagination occurs bilaterally and is considered the start of the vaginal process. Some authors consider this phenomenon to be "active", involving the invasion of the gubernaculum by mesothelial cells (19), while others advocate that this phenomenon is "passive" and secondary to the increase in intra-abdominal pressure (1, 19).

The growth of the vaginal process divides the gubernaculum into three parts: (a) the main gubernaculum, which corresponds to the portion covered by the visceral layer of the peritoneum of the vaginal process; (b) the vaginal gubernaculum, which corresponds to the portion that externally surrounds the parietal portion of the vaginal process, and (c) the infra-vaginal gubernaculum, corresponding to the caudal region of the gubernaculum, which has not been invaded by the vaginal process (19).

The maintenance of this undifferentiated mesenchyme along the inguinal canal and scrotum is essential for the downward extension of the vaginal process to occur, during which it follows the pathway created by dilation of the gubernaculum, forming the canal through which the testis will reach the scrotum (1, 19).

The gubernaculum is a cylindrical structure, covered by a peritoneum on all sides except the posterior, where the testicular vessels and vas deferens are situated. Macroscopically, it looks like the Wharton's jelly of the umbilical cord (1, 12). Histologically, it is composed of undifferentiated cells with elongated shape, surrounded by a large quantity of extracellular material, where it is impossible to identify smooth or striated muscle cells except in its distal end and in the peripheral portion (29) (Figure-1).

The different parts of the gubernaculum undergo varied changes during testicular migration. The vaginal and infra-vaginal portions become proportion-

ally longer as the testis starts to descend to the scrotum. At the same time, their diameter increases, a fact considered one of the most important mechanisms for dilating the inguinal canal to allow the testis to pass across the inguinal canal (1).

The gubernaculum's growth is divided into two phases, triggered by different hormonal stimuli (19). In the first, its volume increases and in the second it decreases in size, coinciding with the complete descent of the testis (1). The cremaster muscle presents structural alterations during this period as well (30, 31). This muscle allows rhythmic contraction to guide the testis into the scrotum in rats and in humans, leading to eversion of the distal portion of the gubernaculum and contributing to its migration to the scrotum (30).

The first phase is marked by pronounced cell multiplication and accumulation of glycosaminoglycans, mainly hyaluronic acid. These substances act as hydrophilic agents and raise the quantity of water. There is also an increase in the amount of extracellular material, explaining the low cell density found at some points (1). The presence of myoblasts intensifies and there are changes in the number and arrangement of the collagen fibers and alterations of the elastic system.

In the second phase, the gubernaculum shrinks, particularly its length, normally accompanied by descent of the testis. This phenomenon appears to be androgen-dependent and brings substantial degradation of the glycosaminoglycans previously accumulated in the extracellular material, with consequent dehydration of this space and condensation of the gubernaculum (20). Although no estimates are available of the degree of shortening, some authors believe this acts together with other factors, causing the gubernaculum to convey the testis to the scrotum (1, 19).

Understanding the relationship between regression of the gubernaculum and descent of the testis is vital to comprehension of how androgens control testicular migration. Studies have demonstrated an association between androgen deficiency, on the one hand, and failed regression of the gubernaculum and cryptorchidism on the other. In this situation, the gubernaculum appears to act as an obstacle to testicular descent (6, 30).

The gubernaculum has its own nerve supply, the GFN, descending on the anterior and medial surface of the psoas muscle from L1-L2 segments (1, 9, 30). The 2nd phase of testicular descent is regulated by androgens and calcitonin gene-related peptide, released from the sensory nucleus of the GFN (5, 15). In rodents, the active proliferation of the gubernacular tip and cremaster muscle, the muscle's rhythmic contraction, and the chemotactic gradient provided by the CGRP together result in migration of the testes into the scrotum. The importance of this mechanism is corroborated by experimental models where the sectioning of the genitofemoral nerve leads to cryptorchidism (12, 23).

The gubernaculum's growth is divided into two phases, triggered by different hormonal stimuli (8, 19). In the first, its volume increases and in the second it decreases in size, coinciding with the complete descent of the testis (28). The cremaster muscle presents structural alterations during this period as well (31). This muscle allows rhythmic contraction to guide the testis into the scrotum in rats and in humans, leading to eversion of the distal portion of the gubernaculum and contributing to its migration to the scrotum (31).

The first phase is marked by pronounced cell multiplication and accumulation of glycosaminoglycans, mainly hyaluronic acid. There is also an increase in the amount of extracellular material, explaining the low cell density found at some points (32, 33). In the second phase, the gubernaculum shrinks, particularly its length, normally accompanied by descent of the testis. This phenomenon appears to be androgen-dependent and brings substantial degradation of the glycosaminoglycans previously accumulated in the extracellular material, with consequent dehydration of this space and condensation of the gubernaculum (29). Although no estimates are available of the degree of shortening, some authors believe this acts together with other factors, causing the gubernaculum to convey the testis to the scrotum (1, 19). The connective tissue of the gubernaculum undergoes remodeling, so that at the end of migration it has essentially become a fibrous structure, rich in collagen and elastic tissue (29).

In a previous study with fetuses without anomalies and fetuses with Prune Belly syndrome the nerves

of gubernaculum testis were analyzed (34, 35). Prune belly syndrome (PBS) is a disorder characterized by deficiency or hypoplasia of the abdominal muscles and/or malformation of the urinary tract, such as large and hypotonic bladders, dilated and tortuous ureters and bilateral cryptorchidism (36, 37). The main pathogenic theory of SPB is urethral obstruction that would cause distension of the urinary tract, preventing the normal development of the abdominal musculature and the descent of the testicles (36). Recently, important alterations in the gubernaculum testis structure were demonstrated in fetuses with PBS (20). Bilateral cryptorchidism is characteristic of prune belly syndrome (36, 37). The contraction of the muscles of the abdominal wall, growth of the liver and intestines and accumulation of meconium all increase the pressure inside the fetal abdomen. According to several authors, this favors testicular migration (23). Contraction of the abdominal musculature is impaired in PBS. Mechanical obstruction due to bladder distention is another factor believed to hinder testicular migration in this syndrome (37). Another theory put forward to explain bilateral cryptorchidism in PBS is the structural alteration of the inguinal canal, which hampers the passage of the testis (37).

In this study about the gubernaculum in PBS with human fetuses (20), we observed a small quantity of nerves both in the gubernaculums of the control group and those of the PBS group (mean of 3.158%) without statistical differences (Figure-1). This is the first study assessing and quantifying the distribution of the nerves of the human testicular gubernaculum. The small quantity of nerves presents in the gubernaculums studied could confirm the theory that the rhythmic contraction of the gubernaculum, mediated by stimulus from the genitofemoral nerve (7, 8, 20, 35), has little importance in humans, but future researches will be necessary to clarify this topic.

The gubernaculums analyzed from the fetuses with PBS showed alterations in the concentrations of collagen and elastic fibers. We did not observe the processus vaginalis developing inside the gubernaculum in Prune Belly Syndrome. These structural alterations could be one of the factors involved in cryptorchidism in prune belly syndrome. The tissue changes in the gubernaculum testis during the fetal period suggest that it plays an active role in testicular migration.

Genitofemoral Nerve

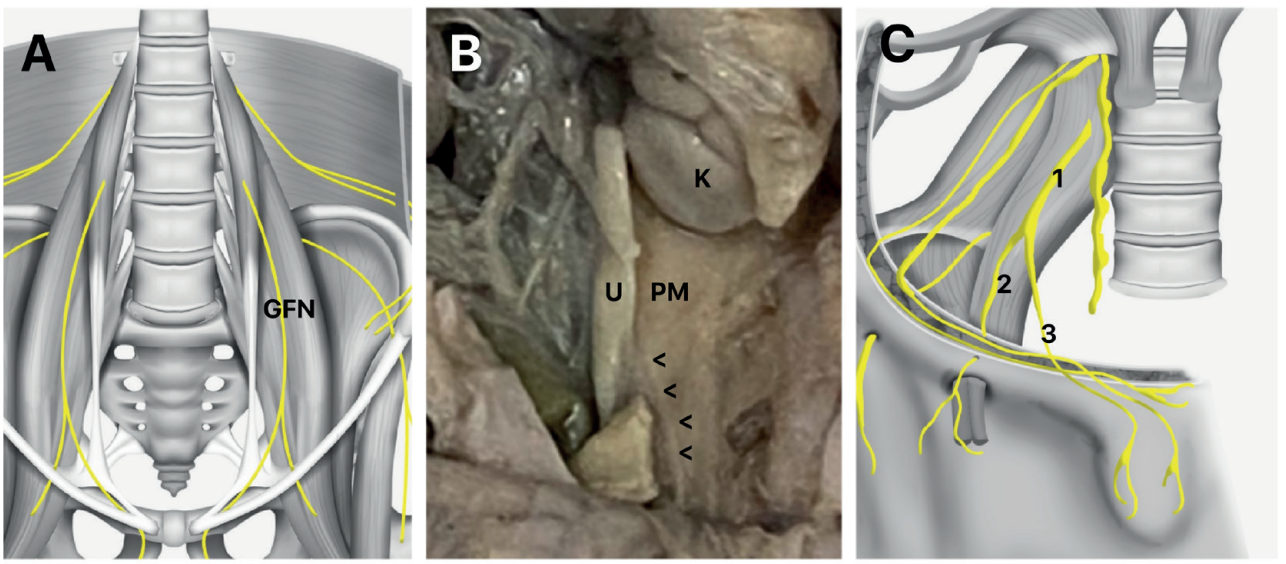
The genitofemoral nerve is a mixed nerve that originates from the lumbar plexus, specifically from the ventral rami of the L1 and L2 spinal nerves. It has both sensory and motor components. Anatomically, the genitofemoral nerve divides into two branches: Genital branch and femoral branch (38, 39) (Figure-2).

The genital branch supplies sensory innervation to the skin of the upper anterior thigh and the skin of the genital region, including the scrotum in males and the mons pubis and labia majora in females. In males, it also innervates the cremaster muscle, which is involved in the regulation of testicular position (38, 39). The femoral branch innervates the femoral triangle, supplying sensory fibers to the skin over the femoral artery and vein, as well as the iliac lymph nodes. Overall, the GFN plays a crucial role in providing sensory innervation to the genital and upper thigh regions, as well as motor innervation to the cremaster muscle in males (Figure-2) (38, 39).

The GFN mainly provides sensory innervation to the genital region and motor innervation to the cremaster muscle, which helps regulate the position of the testes within the scrotum but doesn't directly influence their descent (1, 5). The GFN doesn't directly play a role in testicular migration but there is a theory that shows a regulatory function of this nerve in hormonal action during this process (1, 5).

According to this theory, fetal androgens masculinize the spinal nucleus of the GFN and then the nerve itself (27). Testosterone appears to play an active role in testicular migration, inducing the development of important structures for testicular migration such as the vaginal process, the vas deferens, the epididymis, the inguinal canal and the scrotum. Another mechanism of action of testosterone would be through stimulation of the genitofemoral nerve, which would induce the production of calcitonin gene-related peptide (CGRP) that acts by stimulating the development of the testicular gubernaculum. This masculinization results in an increase in the number of motoneurons in this re-

Figure 2 - Genitofemoral Nerve Anatomy



A) The figure shows a schematic drawing of the genitofemoral nerve (GFN) and its relationship with the psoas major muscle; B) The figure shows a fetus with 22 WPC, the abdomen was dissected and we can observe the GFN (arrowheads) and the relationship of the nerve with the psoas major muscle (PM), U=Ureter, K=Left kidney; and C) The figure shows a schematic drawing of the genitofemoral nerve (1) ramification in femoral branch (2) and in the genital branch (3).

gion with consequent increase in secretion of the calcitonin gene-related peptide (CGRP) (Figure-3). This mechanism was studied in experimental models where the sectioning of the GFN leads to cryptorchidism (1). Increased CGRP levels lead to a rhythmic contraction of the testicular gubernaculum that would induce its migration to the scrotum (5).

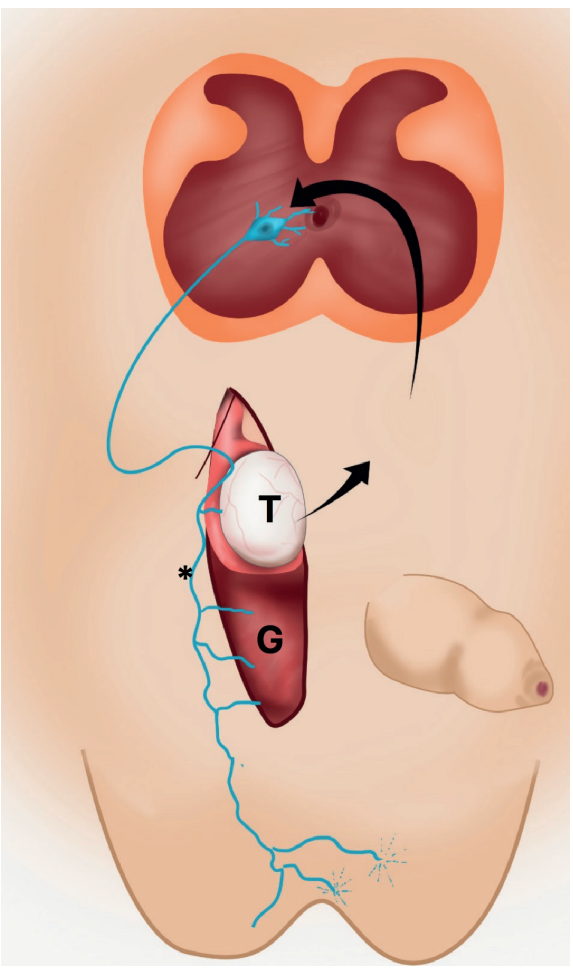
Fetal and placental gonadotropins are also implicated in the process of testicular migration. These substances act by stimulating the production of testicular androgens, which induce the growth and development of the vas deferens, the epididymis, the vaginal process and the gubernaculum itself (8). It is well known that treatment of cryptorchidism with gonadotropins induces testicular migration at levels ranging from 25 to 55% of cases (28). Another endocrine substance involved in testicular migration would be descine (10). This androgen-independent secreted substance in the testis would play an important role in the growth of the gubernaculum mesenchymal cells. The gubernaculum would therefore be one of the fetal structures implicated in testicular migration, most modified by hormonal action (30).

The site of action of the CGRP is the neuromuscular junction. In experimental animals such as rodents, for example, there is an abundance of musculature, fortifying this hypothesis (5), but the human gubernaculum is basically composed of an abundant extracellular matrix with high concentrations of glycosaminoglycans (12, 29), so this theory of CGRP-induced traction in humans is debatable. The indirect action of androgen via the GFN required for testicular descent may be one of the sites of anomalies in the putative multifactorial cause of cryptorchidism (40).

Calcitonin Gene-Related Peptide Gene

The Calcitonin Gene-Related Peptide (CGRP) gene does not have a direct role in testicular migration. CGRP is primarily known for its involvement in vasodilation, neurotransmission, and pain modulation in the peripheral and central nervous systems. It is produced by sensory nerves and is involved in various physiological processes, including regulation of blood flow and inflammation. Testicular migration, on the other hand, is primarily regulated by hormonal factors, such as testosterone and INSL3, and the gubernaculum (1, 9).

Figure 3 - The figure shows a schematic drawing fetal of the genitofemoral nerve (*) action in testicular migration. The androgens originate in testis (T) masculinize the spinal nucleus of the genitofemoral nerve and result in an increase in the number of motoneurons in this region with consequent increase in secretion of the calcitonin gene-related peptide (CGRP) that induces structural modifications of gubernaculum testis (G) during testicular descent.



Insulin-like peptide 3 (INSL3) is a small peptide hormone of the insulin-relaxin family, which is produced and secreted by the fetal Leydig cells in the testes only following gonadal sex determination around 8WPC and represents a major secretory product uniquely from the male fetus (41). The INSL3 is very important during testicular migration because it influences gubernacular ligament thickening, anchoring the testis in the inguinal region and promoting the migration of the testis until the inner inguinal ring (41).

The integrity of the axis between the testis, hypothalamus and pituitary, which regulates testosterone production, is important for the testicular migration process. Cryptorchidism is a common event in pathologies on this axis, such as hypogonadotropic hypogonadism and 5-alpha reductase deficiency (15, 23). Testosterone appears to play an active role in testicular migration, inducing the development of important structures for testicular migration such as the vaginal process, the vas deferens, the epididymis, the inguinal canal and the scrotum. Another mechanism of action of testosterone would be through stimulation of the genitofemoral nerve, which would induce the production of CGRP that acts by stimulating the development of the testicular gubernaculum.

The gubernaculum undergoes a "swelling reaction" during the transabdominal phase and is mainly under the control of INSL-3 and Mullerian Inhibitory Substance/Anti-Mullerian Hormone (1, 9). The 2nd phase of testicular descent is regulated by androgens and calcitonin gene-related peptide (CGRP) release from the sensory nucleus of the GFN. In rodents, the active proliferation of the gubernacular tip and cremaster muscle, its rhythmic contraction, as well as the chemotactic gradient provided by the CGRP result in eventual migration of the testis into the scrotum. Cremaster muscle matures slower than other body muscles, and the persistence of immature myogenic proteins seen in cardiac muscle allows rhythmic contraction to guide the testis into the scrotum. Finally, remodelling of the cremaster muscle enables gubernacular eversion (1, 42, 43). Further understanding of the molecular regulators governing the structural and hormonal changes in the cremaster muscle may lead to new advances in the treatment of undescended testes.

While CGRP may indirectly influence some aspects of reproductive physiology, its role in testicular migration specifically is not well defined.

CONCLUSIONS

The gubernaculum testis has important structural alterations during the testicular migration and the

genitofemoral nerve and CGRP gene are of great importance in this process. In the first phase of testicular migration, the gubernaculum enlarges to hold the testis near the groin and in the second phase the gubernaculum migrates across the pubic region to reach the scrotum. The genitofemoral nerve provides motor innervation to the cremaster muscle and gubernaculum, which helps regulate the position of the testes within the scrotum.

ABBREVIATIONS

GFN = Genitofemoral nerve
CGRP = calcitonin gene-related peptide
WPC = week post-conception
INSL3 = insulin-like factor 3

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CONFLICT OF INTEREST

None declared.

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Correspondence address:
Luciano Alves Favorito, MD, PhD
Unidade de Pesquisa Urogenital, Departamento de Anatomia da Univer. do Est. do Rio de Janeiro – UERJ
Rua Professor Gabizo, 104/201
Rio de Janeiro, RJ, 20271-320, Brasil
Telephone: +55 21 2264-4679
E-mail: lufavorito@yahoo.com.br

REVIEW ARTICLE

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From pathophysiology to practice: addressing oxidative stress and sperm DNA fragmentation in Varicocele-affected subfertile men

Filipe Tenório Lira Neto [1, 2, 3], Lucas Ribeiro Campos [4], Matheus Roque [5], Sandro C. Esteves [6, 7, 8]

[1] Andros Recife, Recife, PE, Brasil; [2] Departamento de Urologia, Instituto de Medicina Integral Prof. Fernando Figueira, Recife, PE, Brasil; [3] Hospital Santa Joana Recife, Recife, PE, Brasil; [4] Departamento de Urologia, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil; [5] Departamento de Medicina Reprodutiva, Mater Prime, São Paulo, SP, Brasil; [6] ANDROFERT - Clínica de Andrologia e Reprodução Humana, Centro de Referência. em Reprodução Masculina, Campinas, SP, Brasil; [7] Departamento de Cirurgia, Disciplina de Urologia da Universidade Estadual de Campinas - UNICAMP, Campinas, SP, Brasil; [8] Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark

ABSTRACT

Varicocele can reduce male fertility potential through various oxidative stress mechanisms. Excessive production of reactive oxygen species may overwhelm the sperm's defenses against oxidative stress, damaging the sperm chromatin. Sperm DNA fragmentation, in the form of DNA strand breaks, is recognized as a consequence of the oxidative stress cascade and is commonly found in the ejaculates of men with varicocele and fertility issues. This paper reviews the current knowledge regarding the association between varicocele, oxidative stress, sperm DNA fragmentation, and male infertility, and examines the role of varicocele repair in alleviating oxidative-sperm DNA fragmentation in these patients. Additionally, we highlight areas for further research to address knowledge gaps relevant to clinical practice.

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Filipe Tenorio Lira Neto
https://orcid.org/0000-0002-6783-307X

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INTRODUCTION

Varicocele is the abnormal enlargement of the veins within the pampiniform plexus due to venous blood reflux caused by incompetent venous valves (1, 2). The condition affects around 15% of the adult male population and 35% of men with primary infertility (3-5). Its incidence rises to 80% among men with secondary infertility, suggesting progressive damage of spermatogenesis (3). This hypothesis is further supported by the progressive impairment of semen analysis (SA) parameters in men with untreated varicocele (6). Most studies involving infertile men with varicocele have shown that the condition impairs SA parameters, such as sperm concentration, sperm motility, and sperm morphology (7-9). Conversely, it is estimated that 80% of the men with varicocele are fertile, making the association between varicocele and decreased semen quality controversial in fertile males (7-10).

Traditionally, the damage to reproductive function caused by varicocele is attributed to testicular hyperthermia due to the loss of the countercurrent mechanism that keeps the testicular temperature 2oC below the core temperature (11). However, recent studies have demonstrated that several non-mutually exclusive factors, including excessive oxidative stress (OS), are implicated in the pathophysiology of varicocele (1).

Currently, varicocele repair is recommended for infertile men with clinical varicocele and abnormal basic SA parameters (12). An abnormal basic semen analysis is defined by alterations in classic parameters like sperm concentration, total sperm count, total motility, progressive motility, normal forms, and vitality (13). Clinical varicocele is defined as a dilation of the pampiniform plexus, either palpable or visible during the physical examination with the patient standing (14). Varicoceles are graded using the criteria of Dubin and Amelar as absent - no palpable varicocele, grade 1 – palpable only with Valsalva maneuver, grade 2 - palpable without Valsalva, and grade 3 - visible (15). The term "subclinical varicocele" is used when the varicocele is not palpable, even with the Valsalva maneuver, but detected by imaging studies, such as the color Doppler scrotal ultrasound (16). Based on these definitions, the treatment of clinical varicoceles in infertile men has been consistently associated with SA parameters, reduced oxidative stress, higher pregnancy rates, and better outcomes in assisted reproductive technology (ART) (4, 17-20). Additionally, improved reproductive outcomes after varicocelectomy have been linked to reduced OS and sperm DNA fragmentation (21, 22 , 23), suggesting that elevated sperm DNA fragmentation levels should be an indication for varicocele repair (24).

In this paper, we review the current knowledge regarding the association between varicocele, oxidative stress, sperm DNA fragmentation, and male infertility, and examine the role of varicocele repair in alleviating oxidative-sperm DNA fragmentation in these patients. We also highlight areas for further research to address knowledge gaps relevant to clinical practice.

Mechanisms of Varicocele-Induced Oxidative Stress

Reactive oxygen species (ROS) are reactive chemical intermediates with one or more unpaired electrons that quickly react with organic compounds to stabilize their electronic structures (25). While primarily viewed as toxic agents, ROS are necessary for normal sperm function. A small degree of lipid peroxidization of the sperm membrane enhances the ability of sperm to bind to the zona pellucida (26). Additionally, small amounts of superoxide, the primary free radical, have been shown to induce hyperactivation and capacitation of human sperm (27). However, OS occurs when excessive ROS are produced, surpassing the antioxidant mechanisms. Unchecked lipid peroxidation and other reduction reactions cause alterations in nuclear and mitochondrial sperm DNA, such as base modification, strand breaks, and chromatin cross-links (28, 29). Due to the limited capacity of spermatozoa to repair its membrane and genetic material, these damages ultimately lead to apoptosis and defective sperm function (30-34).

ROS can be measured using direct or indirect methods (35). Indirect techniques assess by-products of oxidation, such as lipid peroxidation (MDA), protein oxidation (like protein carbonyl), and oxidized DNA (8-hydroxy-2′-deoxyguanosine[8-OHdG]). Direct oxidative stress measurements include total or specific ROS levels in semen and total antioxidant capacity (TAC) (36).

Varicocele has been consistently associated with OS and decreased seminal antioxidant capacity (Table-1). This association is more substantial when

Table 1 - Characteristics of the studies assessing the effect of varicocele on seminal oxidative stress.

Author, year, (country)	Assays	Study groups	Main results
Hendin et al., 1999 (USA) (49)	Seminal ROS by chemiluminescence with luminol; seminal TAC by enhanced chemiluminescence	17 normozoospermic men without varicocele; 15 men with incidental varicocele; 21 infertile men with palpable varicocele	**ROS levels:** Controls: 1.3 ± 0.33 log[ROS+1] Men with incidental varicocele: 1.99 ± 0.26 log[ROS+1] (P<0.05 *versus* controls); Infertile men with varicocele:2.18 ± 0.25 log[ROS+1] (P<0.05 *versus* controls); **TAC:** Controls: 1443.0 ± 105.0 molar Trolox; Men with incidental varicocele: 939.0 ± 107.0 molar Trolox (P<0.05 *versus* controls); Infertile men with varicocele: 1186.0 ± 96.9 molar Trolox (P<0.05 *versus* controls)
Sharma et al., 1999 (USA) (43)	Seminal ROS by chemiluminescence with luminol; seminal TAC by enhanced chemiluminescence	25 normozoospermic fertile, healthy men; 55 infertile men with palpable varicocele	**ROS levels:** Controls: 1.39 ± 0.73 log[ROS+1]; Infertile men with varicocele: 2.10 ± 1.21 log[ROS+1] (P<0.05 *versus* controls) **TAC:** Controls: 1650.93 ± 532.22 molar Trolox; Infertile men with varicocele: 1100.11 ± 410.13 molar Trolox (P<0.05 *versus* controls)
Köksal et al., 2000 (Turkey) (56)	Intratesticular MDA by thiobarbituric acid reaction	10 infertile men without varicocele; 15 infertile men with palpable varicocele	**MDA levels:** Infertile men without varicocele: 33.5 ± 18.93 pmol/mg; Infertile men with varicocele: 38.3 ± 22.92 pmol/mg (P NS *versus* controls); MDA levels in men with grade III varicocele were higher than in men with lower grade varicocele (P<0.05)
Pasqualotto et al., 2000 (USA) (40)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by enhanced chemiluminescence	19 normozoospermic men without varicocele; 77 infertile men with palpable varicocele	**ROS levels:** Controls: 1.3 ± 0.3 log[ROS+1]; Infertile men with varicocele: 2.2 ± 0.13 log[ROS+1] (P<0.05 *versus* controls) **TAC:** Controls: 1653.98 ± 115.29 molar Trolox; Infertile men with varicocele: 1173.05 ± 58.07 molar Trolox (P<0.05 *versus* controls)
Saleh et al., 2003 (USA) (44)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by enhanced chemiluminescence	16 fertile men without varicocele; 15 infertile men without varicocele; 16 infertile men with palpable varicocele	**ROS levels:** Controls: 0.36 (IQR: 0.1, 2) (cpm)/20x106 sperm/mL; Infertile men without varicocele: 1.7 (IQR: 0.1, 5.4) (cpm)/20x106 sperm/mL (P NS *versus* controls); Infertile men with varicocele: 12 (IQR: 1.3, 53.4) (cpm)/20x106 sperm/mL (P<0.05 versus controls) **TAC:** Controls: 871 (IQR: 699, 1288) molar Trolox; Infertile men without varicocele: 904 (IQR: 693, 978) molar Trolox (P NS *versus* controls); Infertile men with varicocele: 693 (IQR: 499, 822) molar Trolox (P<0.05 *versus* controls)

Allamaneni et al., 2004 (USA) (57)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by enhanced chemiluminescence	46 infertile men with palpable left varicocele	Median ROS level 119 (13, 2475) x10^4cpm ROS levels positively correlated with varicocele grade
Mehraban et al., 2005 (Iran) (37)	Seminal total nitrite and nitrate levels	40 fertile men without varicocele; 40 infertile men without varicocele; 40 infertile men with palpable varicocele	Seminal total nitrite and nitrate levels: Controls: 37.06 ± 20.39 μmol/L; Infertile men without varicocele: 33.7 ± 18.99 μmol/L (P NS *versus* controls); Infertile men with varicocele: 52.34 ± 26.62 μmol/L (P<0.05 versus controls and infertile men without varicocele)
Smith et al., 2006 (Chile) (98)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by enhanced chemiluminescence	25 normozoospermic healthy donors 37 men with varicocele and normal SA; 18 men with varicocele and abnormal SA	**ROS levels:** Controls: 2.8 ± 0.9 log[ROS+1]; Men with varicocele and normal SA: 3.3 ± 1.2 log[ROS+1] (P<0.05 *versus* controls); Men with varicocele and abnormal SA: 4.3 ± 1.1 log[ROS+1] (P<0.05 *versus* controls) **TAC:** Controls: 1.2 ± 0.1 mM Trolox; Men with varicocele and normal SA: 1.1 ± 0.4 mM Trolox (P NS *versus* controls); Men with varicocele and abnormal SA: 1.1 ± 0.5 mM Trolox (P NS *versus* controls)
Ishikawa et al. 2007 (Japan) (58)	Intratesticular 8-OHdG positive cell by immunostaining	5 healthy fertile men; 36 infertile men with palpable varicocele and abnormal SA	Incidence of 8-OHdG immunostained germ cells: Controls: 29 ± 5.4%; Varicocele grade I:38 ±10%, (P<0.05 *versus* controls); Varicocele grade II: 41 ± 9.1% (P<0.05 *versus* controls); Varicocele grade III: 57 ± 9.3% (P<0.05 versus controls and grade I+II)
Sakamoto et al., 2008 (Japan) (38)	Seminal NO levels; Seminal 8-OHdG levels; Seminal SOD activity	15 normozoospermic men without varicocele; 15 infertile men with varicocele and normal SA; 15 infertile men with palpable varicocele and oligozoospermia	**NO levels:** Controls: 8.2 ± 4.3μmol/L; Men with varicocele and normal SA:15.4 ± 0.3μmol/L (P<0.05 *versus* controls); Men with varicocele and oligozoospermia: 7.8 ± 4.0 μmol/L (P NS versus controls); **8-OHdG levels:** Controls: 14.7 ± 8.3 μmol/L; Men with varicocele and normal SA:10.0 ± 5.4 μmol/L (P NS *versus* controls); Men with varicocele and oligozoospermia: 10.8 ± 7.5 μmol/L (P NS *versus* controls); **SOD activity:** Controls: 75.6 ± 13.1%; Men with varicocele and normal SA: 84 ± 6.7% (P<0.05 *versus* controls); Men with varicocele and oligozoospermia: 89.4 ± 4.4 % (P<0.05 *versus* controls)
Mostafa et al., 2009 (Egypt) (47)	Seminal MDA by thiobarbituric acid reaction; Seminal H2O2 by spectrophotometric Method; Seminal SOD; Seminal GPx; Seminal Cat	45 fertile men without varicocele; 45 fertile men with varicocele; 42 infertile men with unilateral palpable varicocele and abnormal SA; 44 infertile men with abnormal SA and without varicocele	MDA and H2O2 were significantly higher, and antioxidants were significantly lower in fertile men with varicocele, OA men with and without varicocele compared with controls; All ROS parameters were increased, and all antioxidants were decreased in infertile men with varicocele compared to infertile men without varicocele

Abd-Elmoaty et al., 2010 (Egypt) (62)	Seminal NO levels by colorimetric method; Seminal MDA by colorimetric method; Seminal SOD; Seminal GPx; Seminal Cat	18 fertile men without varicocele; 42 infertile men with varicocele	**MDA levels:** Controls: 8.4 ± 1.3 pmol/mL; Infertile men with varicocele: 13.5 ± 2.8 pmol/mL (P<0.05 *versus* controls) **NO levels:** Controls: 11.3 ± 1.0 nmol/L; Infertile men with varicocele: 17.9 ± 4.1 nmol/L (P<0.05 *versus* controls); CAT, SOD, GPX, and ascorbic acid were significantly lower in infertile men with varicocele compared with fertile men (P values <.05, .01, .01, and .05, respectively)
Blumer et al., 2011 (Brazil) (165)	Seminal MDA by thiobarbituric acid reaction	19 men without varicocele; 12 men with varicocele (fertility status not informed);	**MDA levels:** Controls: 301.4 ± 95.9 ng/mL Men with varicoceles: 287.1 ± 127.7 ng/mL (P NS *versus* controls)
Mostafa et al., 2012 (Egypt) (59)	Seminal MDA by thiobarbituric acid reaction; Seminal H2O2 by spectrophotometric Method; Seminal SOD; Seminal GPx; Seminal Cat	20 fertile men without varicocele; 22 infertile men with grade 1varicocele; 43 infertile men with grade II varicocele; 23 infertile men with grade III varicocele	Levels of MDA and H2O2 were increased and antioxidants; SOD, Cat, GPx, vit.C levels were decreased in men with varicocele of all grades (I, II, III) compared with the controls; Men with grade II and III varicocele demonstrated higher MDA and H2O2 levels as well as decreased activities of SOD, Cat, GPx,and levels of vit.C compared with men with grade I varicocele.
Mostafa et al., 2016 (Egypt) (50)	Seminal MDA by thiobarbituric acid reaction; Seminal GPx	24 fertile men without varicocele; 22 fertile men with varicocele; 34 infertile men with palpable varicocele and abnormal SA; 24 infertile men with abnormal SA and without varicocele	**MDA levels:** Controls: 1.2 ± 0.17 nmol/mL; Fertile men with varicocele: 1.9 ± 0.69 nmol/mL (P<0.05 *versus* controls); Infertile men without varicocele and abnormal SA: 2.4 ± 0.47 nmol/mL (P<0.05 *versus* controls and other groups); Infertile men with varicocele and abnormal SA: 3.02 ± 0.47 nmol/mL (P<0.05 *versus* controls and other groups) **GPx activity:** Controls: 0.47 ± 0.6 U/mL; Fertile men with varicocele: 0.36 ± 0.8 U/mL (P<0.05 *versus* controls); Infertile men without varicocele and abnormal SA: 0.3 ± 0.03 U/mL (P<0.05 *versus* controls and other groups); Infertile men with varicocele and abnormal SA: 0.21 ± 0.04 U/mL (P<0.05 *versus* controls and other groups)
Ni et al., 2016 (China) (61)	Seminal MDA by thiobarbituric acid reaction	25 healthy normozoospermic men without varicocele; 15 infertile men with subclinical varicocele; 19 infertile men with grade I varicocele; 18 infertile men with grade II varicocele; 14 infertile men with grade III varicocele	**MDA levels:** Control group: 7.45 ± 3.58 nmol/mL; Varicocele subclinical group: 7.22 ± 3.33 nmol/mL; Varicocele grade I group: 12.18 ± 4.86 nmol/mL (P<0.05 *versus* controls); Varicocele grade II group: 14.12 ± 5.42 nmol/mL (P<0.05 *versus* controls); Varicocele grade III group: 15.86 ± 6.78 nmol/mL (P<0.05 *versus* controls)

Abdelbaki et al., 2017 (Egypt) (42)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by Colorimetric assay kit	20 normozoospermic fertile men without varicocele; 60 infertile men with palpable varicocele	**ROS levels:** Controls: 2.62 ± 0.8 log[ROS+1]; Infertile men with varicocele: 4.49 ± 0.9 log[ROS+1] (P<0.05 *versus* controls); **TAC:** Controls: 1.5 ± 0.5 mM Trolox; Infertile men with varicocele: 0.97 ± 0.4 mM Trolox (P<0.05 *versus* controls)
Alkan et al., 2018 (Turkey) (60)	Seminal ROS by chemiluminescence with luminol; Seminal superoxide anion by chemiluminescence with lucigenin	13 normozoospermic men without varicocele; 17 men with grade II varicocele; 17 men with grade III varicocele	**ROS levels:** Controls: 2.4 ± 0.1 log[ROS+1]; Men with grade II varicocele: 2.7 ± 0.4 log[ROS+1] (P<0.05 versus controls); Men with grade III varicocele: 3.2 ± 0.5 log[ROS+1] (P<0.05 *versus* controls and grade II group); **Superoxide anion levels:** Controls: 2.3 ± 0.2 log[ROS+1]; Men with grade II varicocele: 2.5 ± 0.3 log[ROS+1] (P<0.05 *versus* controls); Men with grade III varicocele: 3.0 ± 0.5 log[ROS+1] (P<0.05 versus controls and grade II group)
Tanaka et al., 2020 (Japan) (45)	Seminal ORP measured by MiOXSYS	102 normozoospermic men without varicocele; 138 infertile men with palpable varicocele	**ORP:** Men without varicocele: 9.82 ± 10.31 mV/10^6 sperm/mL; Infertile men with varicocele: 16.73 ± 12.13 mV/10^6 sperm/mL (P<0.05 *versus* controls)
Ammar et al., 2021 (Tunisia) (51)	Seminal MDA by thiobarbituric acid reaction; Seminal SOD; Seminal Cat; Seminal GPx	29 fertile men without varicocele; 11 infertile men with palpable varicocele and normal SA; 40 infertile men with palpable varicocele and abnormal SA	**MDA levels:** Controls: 0.56 ± 0.25 nmol/mL; Infertile men with varicocele and normal SA: 1.43 ± 1.2 nmol/mL (P<0.05 versus controls); Infertile men with varicocele and abnormal SA: 1.63 ± 1.38 nmol/mL (P<0.05 *versus* controls); GPx and CAT activities were decreased in both groups with varicocele, and SOD activity was decreased only in infertile men with varicocele and abnormal SA when compared to controls (P<0.05)
Camargo et al., 2021 (Brazil) (166)	Seminal MDA by thiobarbituric acid reaction	15 normozoospermic men without varicocele; 15 infertile men with grade 2 or 3 varicocele	**MDA levels:** Controls: 20.1 ± 4.59 nmol/mL Infertile men with varicocele: 21.6 ± 8.97 nmol/mL (P NS *versus* controls)
Gill et al., 2021 (Poland) (46)	Seminal ORP measured by MiOXSYS	105 normozoospermic men without varicocele; 64 men with proven fertility; 71 infertile men with clinical varicocele; 95 infertile men without varicocele	**ORP:** Normozoospermic group: 1.68 ± 0.91 mV/106 sperm/mL; Proven fertility group: 1.00 ± 0.8 mV/106 sperm/mL; Varicocele group: 36.10 ± 60.97 mV/106 sperm/mL (P < 0.05 versus normozoospermic and proven fertility);

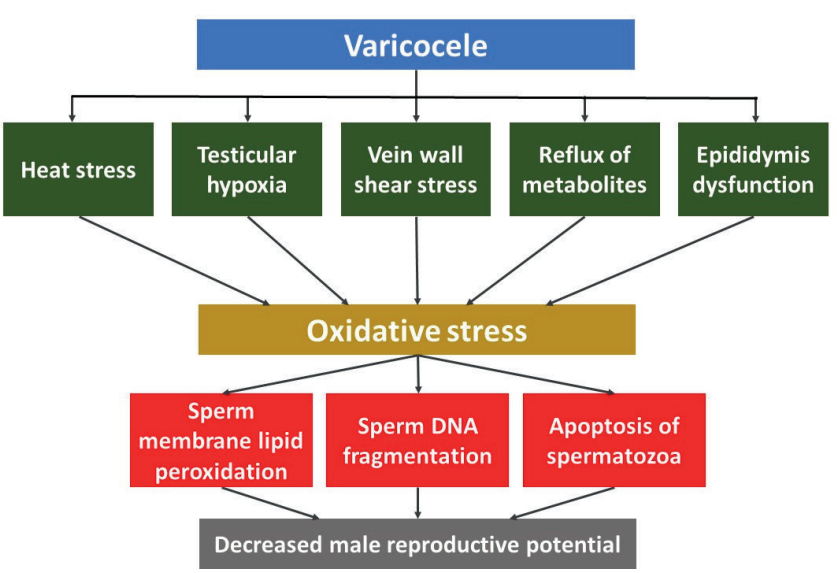
8-OHdG: 8-hydroxy-2′-deoxyguanosine; Cat :catalase activity ; GPx: glutathione peroxidase activity; H2O2: hydrogen peroxide; IQR: interquartile range; MDA: malondialdehyde; NO: Nitric oxide; NS: not significant; ORP: oxidation-reduction potential; ROS: Reactive oxygen species; SA: semen analysis; SOD: superoxide

comparing infertile men with varicocele to fertile men without varicocele (37-46), but even fertile men with varicocele have increased levels of ROS (47-50). Seminal OS biomarkers are found in higher concentrations in infertile men with varicocele, regardless of alterations in basic SA parameters (38, 51). A study by Gill and colleagues involving infertile men with clinical varicocele reported that 83% of these men have elevated OS, measured by oxidation-reduction potential (ORP> 1.37 mV/106 sperm/mL), significantly higher compared to 19% of the men with proven fertility ($P < 0.05$) (46). Moreover, varicocele increases seminal ROS levels as early as adolescence (52).

Varicocele grade has been shown to influence the impairment of basic semen parameters (53, 54). For instance, a large cross-sectional study revealed that semen quality was significantly impaired in men with all varicocele grades, with the most severe impairment at higher grades (55). Higher-grade varicoceles are associated with higher levels of seminal ROS than smaller ones (42, 56-62). In contrast, one study evaluated the impact of subclinical varicocele and did not find increased OS marker levels compared to controls without varicocele (61). Moreover, the only study assessing the influence of varicocele laterality on the severity of OS demonstrated increased expression of cyclooxygenases in infertile men with bilateral varicocele compared to men with unilateral varicocele (50). Thus, it is reasonable to assume that varicocele grade influences the severity of varicocele-induced OS. However, the limited number of studies prevents a definitive conclusion regarding the impact of varicocele laterality and subclinical varicocele on ROS production.

Despite the link between varicocele and OS, the mechanisms underpinning this association are yet to be fully clarified. The most studied effects of varicocele that could increase ROS production or decrease TAC include scrotal hyperthermia, testicular hypoxia, vein wall shear stress, adrenal/renal metabolites reflux, and epididymal response (63) (Figure-1). Additionally, most men with varicocele are fertile; however, the pathways that prevent damage to spermatogenesis in these men are unclear (10). Proposed response mechanisms include increased production of enzymatic and non-enzymatic ROS scavengers such as catalase, superoxide dismutase, vitamin C, and glutathione peroxidase (35, 48, 64).

Figure 1. Pathophysiology of Varicocele and its Association with Sperm DNA



Fragmentation. Lines indicate direct effects. Adapted from Cho et al. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. *Asian J Androl*. 2016 Mar-Apr;18(2):186-93, under the Creative Commons Attribution License terms.

Heat Stress

Scrotal hyperthermia was the first hypothesis described to explain oxidative stress in varicocele (28). The optimal temperature for spermatogenesis is 2 to 4°C lower than the body's average temperature. This difference is maintained by several mechanisms, including the contraction of the cremaster and dartos muscles and the countercurrent system in the pampiniform plexus (65). Incompetent valves of the internal spermatic and cremasteric veins allow venous blood to reflux into the pampiniform plexus, disrupting the countercurrent mechanism and raising the scrotal temperature (66, 67). Heat stress is associated with increased ROS production by several organelles, such as cell mitochondria, plasma membrane, cytoplasm, and peroxisomes (68). The severity of the damage caused by hyperthermia varies among the various cell compartments (30). In the testes, spermatogonia B and the developing spermatozoa are the most susceptible to heat stress, whereas spermatogonia A, Leydig, and Sertoli cells are relatively resistant to hyperthermia (11, 28).

Testicular Hypoxia

Venous reflux hampers normal circulation in the testicular microvessels, leading to testicular ischemia in men with varicocele (69). Using ultra-sensitive Doppler ultrasound to measure testicular flow, Rocher and colleagues reported a decrease by 60% and 80% ($P<0.05$) in arterial blood flow during the Valsalva maneuver in patients with grades 2 and 3 varicoceles, respectively, suggesting that ischemia occurs when the venous hydrostatic pressure of the internal testicular vein exceeds the testicular arteriolar pressure (70). Another study demonstrated that a peak retrograde flow higher than 38 cm/s was linked to increased sperm DNA damage in men with varicocele (71). Arteriolar occlusion by microthrombi and subsequent ischemic alterations, including germ-cell degeneration, Leydig cell atrophy, and fibrotic thickening of the seminiferous tubules' basement membranes, are commonly reported in histopathological analysis of testicular biopsy specimens from infertile men with varicocele (72). This hypoxic state leads to excessive ROS generation from various molecular pathways, including activation of hypoxia-inducible factor 1 (HIF-1), mitochondrial dysfunction, xanthine dehydrogenase/oxidase, membrane-associated NADPH oxidase 5 (NOX5), and phospholipase A2 (28). Furthermore, hypoxia increases the expression of leptin and cytokines in testicular tissue, including interleukin (IL)-1 and IL-6, which also contribute to ROS production (58, 73, 74).

Vein Wall Shear Stress

Varicose veins from patients with chronic venous insufficiency exhibit increased production of ROS and decreased antioxidant potential (75, 76). These studies suggest that the shear stress caused by local hydrostatic hypertension can activate adhesion molecules, such as selectins, integrins, intercellular adhesion molecule 1, and vascular cell adhesion protein 1, leading to increased leukocyte migration to the vein wall. Once migrated, these leukocytes become activated and produce increased amounts of ROS. The shear stress and hypoxic environment in the blood vessels can also induce excessive nitric oxide production via endothelial nitric oxide synthase, further aggravating the oxidative stress in the testicular microcirculation (77).

Reflux of Adrenal/Renal Metabolites

Phlebographic studies have demonstrated retrograde blood flow from renal and adrenal veins to the left testicular vein in men with varicocele (78). Some authors have described the reflux of renal and adrenal metabolites, such as prostaglandins, urea, and adrenomedullin, back to the internal spermatic veins, which could induce cellular OS (79-83).

Epididymis Dysfunction

Animal models of experimental varicocele have demonstrated structural and functional changes in the epididymis, revealing three critical sources of ROS, namely, metabolically active principal cells, endothelial cells from the capillary network around the epididymis caput, and the luminal fluid from the testis (28). These ROS accumulate primarily in the initial epididymal segment. However, the cells from all epididymal sections produce and release antioxidants in the epididymal fluid. Hypoxia and heat stress cause principal cells to generate excessive ROS, which, combined with impaired

antioxidant production, result in oxidative damage to maturing sperm and epididymal cells (30).

Varicocele and Sperm DNA Fragmentation

The WHO cut-off levels for basic SA parameters are poor predictors of natural pregnancy and ART success (13, 84-86). One reason is that routine SA does not include tests to assess sperm function, making it unable to diagnose alterations that could hamper embryo development and implantation (13, 84). Since varicocele is associated with OS, and one of the downstream effects of excessive ROS production is DNA damage, recent studies have focused on markers of DNA damage in assessing varicocele and sperm quality. These biomarkers include chromatin compaction, DNA methylation, and DNA fragmentation (87-90).

Sperm DNA Fragmentation Tests

Several assays detect sperm DNA strand breaks. Some methods use enzymatic reactions to label the strand breaks (e.g., terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; TUNEL), while others use controlled DNA denaturation coupled with protein depletion to reveal the breaks (e.g., sperm chromatin structure assay [SCSA], sperm chromatin dispersion test [SCD], and the Comet assay) (91). A detailed analysis of assays' characteristics is beyond this article's scope and can be found elsewhere (91).

These tests measure the global sperm DNA fragmentation and provide information about sperm quality. Testing should be performed on neat semen samples after a standardized ejaculatory period of 2–3 days, as sperm DNA fragmentation levels increase with prolonged abstinence (92). Although each test detects DNA breaks using different strategies, thresholds of about 20% (by TUNEL, SCSA, SCD, and alkaline Comet) accurately discriminate between fertile and infertile men (93). Moreover, values greater than 20%–30% (by SCSA, alkaline Comet, and SCD) are optimal for classifying infertile couples into a statistical probability of prolonged time to achieve natural pregnancy, decreased likelihood of pregnancy by IUI, IVF, or ICSI and increased risk of miscarriage (91).

The sperm DNA fragmentation tests mentioned above are the most frequently used in clinical practice, and their results have a moderate-to-high correlation (94-96). Supporting these findings, a meta-analysis demonstrated an adverse effect of high sperm DNA fragmentation levels on clinical pregnancy rates after IVF/ICSI, irrespective of the measurement method (i.e., TUNEL, SCSA, SCD, and Comet) (97). Similarly, another meta-analysis demonstrated that the type of test used did not influence the positive effect of varicocelectomy on reducing sperm DNA fragmentation levels (23).

Association Between Varicocele and Sperm DNA Fragmentation

High DNA fragmentation rates are frequently found in infertile men with varicocele (Table-2). Early studies revealed elevated sperm DNA fragmentation levels in infertile patients compared to fertile controls (44, 98). Smith and colleagues reported higher sperm DNA fragmentation levels in men with grade 2 or 3 varicocele than in healthy semen donors (26.1% ± 3.2% vs. 14.2% ± 1.2%, $P<0.05$), even when basic SA parameters were within the WHO reference ranges (98). The authors also demonstrated that a higher proportion of men with palpable varicocele and abnormal basic SA parameters had increased sperm DNA damage than men with varicocele and basic semen parameters within the reference ranges (58% vs. 49%, P-value not reported). This finding suggests that sperm DNA fragmentation levels increase as varicocele damage progresses.

Similarly, Ammar and colleagues reported that infertile men with palpable varicocele displayed increased sperm DNA fragmentation levels regardless of alterations in basic SA parameters; however, sperm DNA damage was greater in those with abnormal SA (51). Moreover, Jeremias and colleagues showed that men with varicocele can present with increased sperm DNA fragmentation even when basic semen analysis parameters are within the WHO reference ranges (99). Conversely, Ni and colleagues assessed sperm DNA fragmentation in infertile men with clinical varicocele and did not find increasing sperm DNA fragmentation rates after six months of observation compared to baseline, despite a worsening in the basic SA parameters (61). Interestingly, using an animal

Table 2. Characteristics of the studies assessing the effect of palpable varicocele on sperm DNA fragmentation

Author, year, (country)	SDF assay	Study groups	Main SDF results
Saleh et al., 2003 (USA) (44)	SCSA	16 fertile men without varicocele, 16 infertile men with palpable varicocele and 15 infertile men without varicocele	Control group: 15.0% (IQR: 10.0%, 22.0%); Infertile with varicocele: 25.0% (IQR: 20.0%, 35.0%) (P < 0.05 *versus* control); Infertile without varicocele: 20.0% (IQR: 13.0%, 28.0%)
Smith et al., 2005 (Chile) (98)	TUNEL and SCSA	25 healthy men without varicocele, 37 men with grade 2 and 3 varicocele and normal SA, 18 men with grade 2 or 3 varicocele and abnormal SA (fertility status not informed)	Control group: TUNEL 14.2% ± 1.2%; SCSA 7.1% ± 0.9%; Varicocele and normal SA group: TUNEL 26.1% ± 3.2%; SCSA 20.7% ± 4.0% (P < 0.05 *versus* control); Varicocele and abnormal SA group: TUNEL 32.2 ± 4.1%; SCSA 35.5% ± 9.0 % (P < 0.05 versus control)
Talebi et al., 2008 (Iran) (167)	SCSA	20 fertile men without varicocele and 20 infertile men with grade 2 or 3 varicocele	Control group: 17.3% ± 7.4%; Varicocele group: 60.5% ± 15.5% (P < 0.05 *versus* control)
Wu et al., 2009 (Taiwan) (168)	Comet	5 healthy men without varicocele and 15 men with grade 2 or 3 varicocele (fertility status not informed)	Control group: 4.5% ± 0.9%; Varicocele group: 8.4% ± 3.1% (P < 0.05 *versus* control)
Blumer et al., 2011 (Brazil) (165)	Comet	19 men without varicocele; 12 men with varicocele (fertility status not informed);	Class II sperm DNA fragmentation: Control: 51.3% ± 14.7 %; Men with varicocele: 59.4% ± 14.8% (P<0.05 *versus* control); There were no differences regarding the other three classes of sperm DNA fragmentation.
La Vignera et al., 2012 (Italy) (154)	TUNEL	30 fertile men without varicocele, 30 infertile men with grade 3 left varicocele and abnormal SA	Control group: 2.0% ± 1.0%; Varicocele group: 5.0% ± 3.0% (P < 0.05 *versus* control)
Li et al., 2012 (China) (169)	SCSA	19 healthy normozoospermic men and 19 infertile men with palpable varicocele and abnormal semen parameters	Control group: 17.4% ± 5.3%; Varicocele group: 28.4% ± 15.6% (P < 0.05 *versus* control)
Esteves et al., 2015 (Brazil) (101)	SCD	80 fertile donors and 98 infertile men with varicocele	Control group: 11.3% ± 5.5%; Varicocele group: 33.5% ± 18,3% (P < 0.05 *versus* control)
Alhathal et al., 2016 (Canada) (170)	SCSA	6 healthy normozoospermic men without varicocele, and 29 infertile men with palpable varicocele and abnormal semen parameters	Control group: 7.4% ± 5.0%; Varicocele group: 20.0% ± 10.6% (P < 0.05 *versus* control)
Ni et al., 2016 (China) (61)	SCSA	25 healthy normozoospermic men without varicocele, 19 infertile men with grade 1varicocele, 18 infertile men with grade 2 varicocele, and 14 infertile men with grade 3varicocele	Control group: 12.0% ± 7.9%; Varicocele grade 1 group: 23.6% ± 7.5% (P < 0.05 *versus* control); Varicocele grade 2 group: 27.7% ± 9.0% (P < 0.05 *versus control*); Varicocele grade 3 group: 30.0 % ± 8.3% (P < 0.05 *versus control*)

Abdelbaki et al., 2017 (Egypt) (42)	SCSA	20 fertile normozoospermic men without varicocele, and 60 infertile men with palpable varicocele and abnormal semen parameters	Control group: 7.6% ± 2.8%; Varicocele group: 29.9% ± 8.3% (P< 0.05 *versus* control)
Dieamant et al., 2017 (Brazil) (171)	TUNEL	2008 men without varicocele and 391 men with palpable varicocele (fertility status not informed)	Control group: 15.3% ± 8.5%; Varicocele group: 16.3% ± 8.8% (P < 0.05 *versus* control)
Santana et al., 2019 (Brazil) (107)	SCD	20 men without varicocele, and 19 men with varicocele (fertility status not informed)	Control group: 26.0% ± 10.0%; Varicocele group: 37.0% ± 20.0% (P = 0.09 *versus* control)
Lara-Cerrillo et al., 2020 (Spain) (172)	Comet	12 fertile men without varicocele and 20 infertile men with grades 2 or 3 varicocele	Control group: 45.0% ± 56.0%; Varicocele group: 53.0% ± 45.0% (P value not informed)
Tanaka et al., 2020 (Japan) (45)	SCSA	102 normozoospermic men without varicocele and 138 infertile men with palpable varicocele	Control group: 9.8% ± 10.3%; Varicocele group: 16.7% ± 12.1% (P < 0.05 *versus* control)
Ammar et al., 2021 (Tunisia) (51)	TUNEL	29 fertile men without varicocele; 11 infertile men with palpable varicocele and normal SA; 40 infertile men with varicocele and abnormal SA	Control group: 8.14% ± 6.86%; Varicocele and normal SA group: 60.87% ± 8.61% (P < 0.05 versus control); Varicocele and abnormal SA group: 69.88% ± 5.87% (P < 0.05 versus control)
Camargo et al., 2021 (Brazil) (166)	Comet	15 normozoospermic men without varicocele; 15 infertile men with grade 2 or 3 varicocele	Control group: 39.3% ± 11.69%; Varicocele group: 43.6% ± 11.9% (P = NS *versus* control)
Gil et al., 2021 (Poland) (46)	SCD	105 normozoospermic men without varicocele; 64 men with proven fertility; 71 infertile men with clinical varicocele; 95 infertile men without varicocele	Normozoospermic group: 13.3% ± 5.9%; Proven fertility group: 13.9% ± 7.1%; Varicocele group: 23.3% ± 11.9% (P < 0.05 versus normozoospermic and proven fertility); Infertile without varicocele: 19.4% ± 5.9% (P < 0.05 versus normozoospermic and proven fertility)
Jellad et al., 2021 (Tunisia) (106)	TUNEL	15 normozoospermic men without varicocele; 30 infertile men with palpable varicocele	Control group: 64.5% ± 17.7%; Varicocele group: 72.0% ± 15.3% (P < 0.05 *versus* control)
Jeremias et al., 2021 (Brazil) (99)	Comet	39 normozoospermic men without varicocele; 55 normozoospermic men with palpable varicocele	Control group: 39.3% ± 11.69%; Varicocele group: 43.6% ± 11.9% (P = NS *versus* control)

SDF: Sperm DNA Fragmentation; %SDF: sperm DNA fragmentation rate; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; SCSA: sperm chromatin structure assay; SCD: sperm chromatin dispersion test; IQR: interquartile range; NS: not significant

model, Carvalho and colleagues observed a negative progressive effect of varicocele on sperm DNA fragmentation (100).

A multicentric study involving 593 men with various causes of infertility found that sperm DNA fragmentation levels were the highest in men with varicocele (35.7% ± 18.3%) and in those with subclinical genital infection (41.7% ±17.6%) compared to a control group of fertile semen donors (11.3% ± 5.5%; P<0.05) (101). Moreover, two separate groups of sperm DNA breaks were identified: standard DNA fragmentation and degraded DNA fragmentation (DDS). Spermatozoa with standard

fragmented DNA exhibited either the absence or presence of a small halo of chromatin dispersion around a compact nucleoid; in contrast, spermatozoa with degraded DNA showed a ghost-like morphology owing to massive single-strand and double-strand DNA breaks in addition to nuclear protein damage. In the study mentioned above, the proportion of sperm with degraded DNA was 8-fold higher in varicocele patients than in donors (54% ± 16% vs. 4.8% ± 7%; P<0.05). Interestingly, despite sperm with degraded DNA not being pathognomonic of varicocele, the index of sperm with degraded DNA reached an accuracy of 94% to discriminate between participants with and without varicocele (101).

Three systematic reviews have confirmed the link between varicocele and sperm DNA fragmentation. The first review by Zini and Dohle was a qualitative analysis of 16 case-control studies assessing sperm DNA fragmentation in fertile and infertile men with and without palpable varicocele (102). In four out of nine studies, sperm DNA fragmentation levels were higher in infertile men with clinical varicocele than in infertile counterparts without varicocele. Furthermore, men with clinical varicocele had worse SA parameters than infertile patients without varicocele. The remaining seven studies specifically included fertile men with clinical varicocele. In six of them, sperm DNA fragmentation rates were higher in men with clinical varicocele (and no history of infertility) than in fertile men or sperm donors without varicocele (102). This review indicates that varicocele not only increases sperm DNA fragmentation rates in men with infertility but also in those with "normal" fertility.

The second systematic review retrieved data from seven studies, including 240 patients with clinical varicocele and 176 controls without varicocele (103). The results revealed that sperm DNA fragmentation was higher in men with varicocele than in controls without varicocele (Mean difference: 9.84%; 95% CI: 9.19–10.49, P<0.05). However, the authors included studies with adolescents and pooled data irrespective of the assay used for sperm DNA fragmentation assessment.

The most recent meta-analysis compiled 12 case-control studies, including 875 participants with clinical and subclinical varicocele and 2377 men without varicocele (104). The authors reported a standard mean difference of 1.40% (95% CI: 0.83%–1.98%, P<0.05) between the groups. A subanalysis by type of sperm DNA fragmentation assay (TUNEL, Comet, and SCSA) revealed increased sperm DNA fragmentation in men with varicocele, irrespective of the assay utilized.

Only two studies looked into the proportion of men with varicocele who have increased sperm DNA fragmentation levels. Abdelaziz and colleagues analyzed a cohort of 54 infertile men with palpable varicocele and reported that 52% of them had sperm DNA fragmentation >30% (measured by TUNEL) (105). Moreover, another study found DNA fragmentation rates >30% in 21% of the infertile men with clinical varicocele, whereas only 1.5% of men with proven fertility demonstrated such high DNA damage, with an odd ratio of 16.8 (46).

Overall, current evidence indicates that men with palpable varicocele have increased sperm DNA fragmentation levels than men without varicocele. The effect is more evident in those men with abnormal basic SA parameters. Remarkably, the results are consistent and do not vary much with the type of test used. Nevertheless, the effect size fluctuated significantly, from 3% to 22%, possibly due to using different assays and the participant characteristics.

Some authors have investigated the influence of varicocele grade on sperm DNA fragmentation. Jellad and colleagues described that varicocele grade was positively associated with sperm DNA fragmentation (15.2% ± 1.9% in grade 3 vs. 12.9% ± 3.5% in grade 2, P<0.05) (106). Similarly, young men (aged 16 to 26) with grade 3 varicocele demonstrated increased sperm DNA fragmentation levels compared to those with grades 1 and 2 (71). Moreover, the study by Ni and colleagues assessed sperm DNA fragmentation in men with palpable varicocele and found numerically higher levels of DNA damage as varicocele grade increased (23.6% ± 7.5% in grade 1, 27.7% ± 9.0% in grade 2, and 30.0% ± 8.3 in grade 3; P value not reported) (61). In contrast, Santana and colleagues reported no differences in sperm DNA fragmentation levels between men with grade 2 and 3 varicoceles (41% ± 24% vs. 34% ± 13%, P=0.99) were reported (107). The only study examining the influence of laterality on sperm DNA damage reported that sperm

DNA fragmentation levels were higher in men with bilateral varicocele than in those with unilateral varicocele (16.4% ± 10.1% vs.12.0% ± 8.8%, P < 0.05) (108). Given the limited data available, further research into this matter is warranted.

There is even less published data about the effect of subclinical varicocele on sperm DNA fragmentation levels (109). García-Peiró and colleagues, using SCD, demonstrated that men with subclinical varicocele have increased sperm DNA fragmentation levels compared to fertile donors (37.5% vs.12.0%, P value not reported) (110). In contrast, Ni and colleagues reported no differences between infertile men with subclinical varicocele and fertile men without varicocele (14.9% ± 5.1% vs.12.0% ± 7.9, by SCSA), even though men with subclinical varicocele had significantly lower basic SA parameters than controls (P<0.05) (61). Additionally, the authors demonstrated that patients with subclinical varicocele had no deterioration of sperm DNA fragmentation levels over a 6-month follow-up (61).

Evidence of the association between varicocele and elevated sperm DNA fragmentation has been increasing steadily (111, 112). Similarly, other markers of sperm function, including epididymal neutral α-glucosidase and sperm PLCζ levels, are also reduced in men with high SDF and grade II or III varicocele (111).

Impact of Varicocele Repair on Oxidative Stress and Sperm DNA Fragmentation

Varicocelectomy Techniques and Rationale

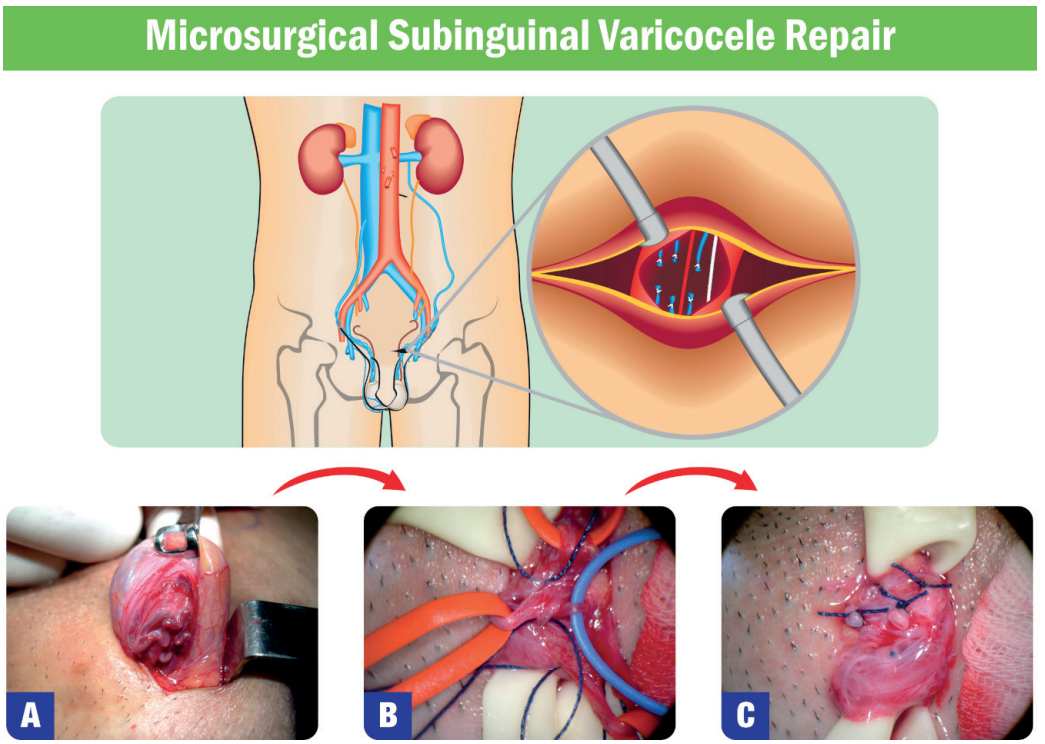
Varicocele repair is typically recommended for infertile men with a palpable disease and abnormal basic SA parameters (113-115) since improvements in basic semen parameters and pregnancy outcomes after varicocelectomy are consistently observed in these individuals (12, 116). Conversely, varicocele repair is not routinely recommended for males with subclinical varicocele due to the contradictory evidence regarding the benefit in this population (117-119).

Surgical repair has been the standard treatment for infertile men with varicocele since Celsus, in the first century A.D., performed the first documented varicocelectomy (120). The main goal of varicocele repair is the occlusion of varicose veins of the pampiniform plexus and their collateral drainage via the external spermatic and cremasteric veins while preserving testicular arteries, lymphatics, and nerves (121, 122). Several techniques have been applied, including open surgical methods (with or without microsurgery), laparoscopy, and embolization. In the open technique, ligation of the veins is performed via retroperitoneal, inguinal, or subinguinal incisions (123). With laparoscopy, the spermatic veins are occluded a few centimeters from the internal inguinal orifice (124). Radiological embolization is carried out via femoral or jugular veins, and the interruption of venous flow through the internal spermatic and collateral veins is achieved using embolic agents (125).

The gold-standard treatment is microsurgical varicocelectomy (MV) (126). Its main surgical steps are illustrated in Figure-2. The improvement rate of basic SA parameters varies from 64 to 81% after MV, and the likelihood of improvement positively correlates with varicocele grade (15, 127-129). Total motile sperm count increases after varicocele repair, which may allow couples needing ICSI to use less invasive assisted conception modalities (e.g., IUI) or attempt natural pregnancy (130). Moreover, the latest Cochrane meta-analysis demonstrated that varicocelectomy increases natural pregnancy rates compared with delayed or no treatment in infertile men with palpable varicocele and abnormal basic SA parameters (RR 1.94, 95% CI 1.23–3.05, P<0.05, seven randomized controlled trials; 693 participants) (131). The authors reported that, on average, six patients would have to undergo varicocelectomy for one additional couple to achieve a natural pregnancy.

There is a broad variation in the natural pregnancy rates after MV, ranging from 29% to 60% during the first 12 months after the procedure (127, 132). Factors such as female infertility, baseline semen parameters, varicocele severity, and other associated male comorbidities impact the reproductive outcomes of MV and contribute to this wide variation. Varicocele repair also increases pregnancy rates by ICSI compared to couples whose male partners did not undergo treatment (clinical pregnancy: OR 1.59, 95% CI 1.19–2.12, P<0.05), four observational studies, 852 participants; live birth: OR 2.17, 95% CI 1.55–3.06, P<0.05, three observational studies, 622 participants) (20). Based on these findings, five patients, on

Figure 2. Microsurgical Varicocele Repair.



A ~2-cm transversal skin incision is made below the external inguinal ring (upper). The muscle layers and the inguinal canal are not violated. The lower panels show intraoperative photographs of the spermatic cord structures. The spermatic cord is exteriorized, and the cremasteric veins are identified and ligated (A). In panel B, the spermatic cord is dissected to allow the identification of the testicular artery (blue vessel loop), dilated varicose veins (red vessel loops), and lymphatics (blue cotton sutures). The testicular artery and lymphatic channels are preserved, whereas the dilated veins are ligated with nonabsorbable sutures and transected (C).

average, would have to receive varicocele repair before ICSI (versus no treatment) for one additional couple to achieve a pregnancy.

Furthermore, microsurgical varicocelectomy increases intratesticular testosterone production, an essential process for normal spermatogenesis (133). A meta-analysis evaluating the impact of varicocele repair on the testosterone levels of hypogonadal men (i.e., having serum total testosterone levels below 300 ng/dL) reported an increase of 123 ng/dL in the total testosterone levels compared to the preoperative levels (P<0.05) (134).

Impact of varicocelectomy on Oxidative Stress

Since varicocele is associated with excessive levels of ROS in the semen, some authors have evaluated the role of varicocele repair in alleviating seminal OS (Table-3). Dada and colleagues demonstrated a decrease in the ROS levels measured by the chemiluminescence method using luminol as a probe in 11 men with clinical varicocele one month after varicocelectomy (preoperative: 142,897.704 RLU per 20 million sperm/min vs. postoperative: 10,776.736 RLU per 20 million sperm/min; P<0.001) (135). The authors also reported a further decrease in ROS levels in men who returned for follow-up after six months of varicocelectomy (135). Similarly, Abdelbaki and colleagues reported reduced ROS levels measured by chemiluminescence and increased TAC in a cohort of 55 men who underwent varicocele repair (42). Furthermore, using seminal MDA measurement to assess ROS levels, Ni and colleagues demonstrated an improvement of OS in infertile men with all three grades of palpable varicocele at 3 and 6 months after MV (61). However, the authors did not find differences in seminal MDA levels between couples who achieved natural

Table 3. Characteristics of the studies assessing the effects of varicocelectomy on oxidative stress

Author, year, (country)	ROS assay	Study groups	Main results
Mancini et al., 2004 (Italy) (140)	Seminal TAC by Colorimetric assay kit	25 infertile men with varicocele 14 infertile men who underwent varicocelectomy 10-24 months before	**TAC:** Preoperative: 106.6 ± 8.9 seconds; Postoperative: 105.8 ± 8.7 seconds (P NS *versus* preoperative)
Sakamoto et al., 2008 (Japan) (38)	Seminal NO levels; Seminal 8-OHdG levels; Seminal SOD activity	Not reported	**NO levels:** Preoperative: 17.1 ± 9.1μmol/L; 6 months postoperative: 7.5 ± 4.5μmol/L (P<0.05 versus preoperative) **8-OHdG levels:** Preoperative: 10.3 ± 4.7 μmol/L; 6 months postoperative: 6.2 ± 2.5 μmol/L (P<0.05 *versus* preoperative) **SOD activity:** Preoperative: 85.8 ± 5.8%; 6 months postoperative: 78.1 ± 8.1% (P<0.05 *versus* preoperative)
Dada et al., 2010 (India) (135)	Seminal ROS by chemiluminescence with luminol	11 infertile men with palpable varicocele	**ROS levels:** Preoperative: 142,897.704 RLU per 20 million sperm/min 1 month postoperative: 10,776.736 RLU per 20 million sperm/min (P < 0.05 *versus* preoperative); 3 months postoperative: 6,456.249 RLU per 20 million sperm/min (P < 0.05 *versus* preoperative)
Baker et al., 2013 (USA)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by Colorimetric assay kit	24 infertile men with palpable varicocele	**ROS levels:** Preoperative: 1185.1 RLU/sec/10^6; 3 months postoperative: 2710.911851 RLU/sec/10^6 (P not reported) **TAC:** Preoperative: 2292 μM Trolox; 3 months postoperative: 1885 mM Trolox (P < 0.05 *versus* preoperative); % patients with TAC above normal: Preoperative: 86%; 3 months postoperative: 71% (P value not reported)
Tavalaee et al., 2015 (Iran) (136)	Seminal OS by DCFH-DA staining	23 infertile men with varicocele grade II and III	**DCFH-DA negative spermatozoa:** Preoperative: 37.2% ± 3.6 %; 3 months postoperative: 61.3% ± 5.3 % (P < 0.05 *versus* preoperative)

Barekat et al., 2016 (Iran) (137)	Seminal OS by DCFH-DA staining	20 infertile men with varicocele grade II and III	**DCFH-DA positive spermatozoa:** Preoperative: 47.6% ± 6.6 %; 3 months postoperative: 36.6% ± 3.8 % (P < 0.05 *versus* preoperative)
Abdelbaki et al., 2017 (Egypt) (42)	Seminal ROS by chemiluminescence with luminol; Seminal TAC by Colorimetric assay kit	55 infertile men with palpable varicocele	**ROS levels:** Preoperative: 4.49 ± 0.9 log[ROS+1]; 3 months postoperative: 3.27 ± 1.3 log[ROS+1] (P < 0.05 *versus* preoperative) **TAC:** Preoperative:1.01 ± 0.4 mM Trolox; 3 months postoperative: 2.05 ± 0.5 mM Trolox (P < 0.05 *versus* preoperative)
Ni et al., 2016 (China) (61)	Seminal MDA by thiobarbituric acid reaction	19 infertile men with grade I varicocele; 18 infertile men with grade II varicocele; 14 infertile men with grade III varicocele	**MDA levels:** Preoperative varicocele grade I group: 12.18 ± 4.86 nmol/mL; 3 months postoperative varicocele grade I group: 9.88 ± 3.98 nmol/mL (P NS *versus* preoperative); 6 months postoperative varicocele grade I group: 8.76 ± 2.73 nmol/mL (P < 0.05 *versus* preoperative); Varicocele grade II group: 14.12 ± 5.42 nmol/mL; 3 months postoperative varicocele grade II group: 9.22 ±3.75 nmol/mL (P < 0.05 *versus* preoperative) 6 months postoperative varicocele grade II group: 9.71 ± 2.83 nmol/mL (P < 0.05 *versus* preoperative); Varicocele grade III group: 15.86 ± 6.78 nmol/mL; 3 months postoperative varicocele grade II group: 11.38 ± 3.94 nmol/mL (P < 0.05 *versus* preoperative); 6 months postoperative varicocele grade III group: 9.50 ± 3.28 nmol/mL (P < 0.05 *versus* preoperative)
Abbasi et al., 2020 (Iran) (138)	Lipid peroxidation by the BODIPY probe	22 infertile men with varicocele grade II and III	**BODIPY-positive spermatozoa:** Preoperative: 36.22% ± 3.38 %; 80 days postoperative: 24.04% ± 1.80 % (P < 0.05 *versus* preoperative)
Kavoussi et al., 2022 (USA) (139)	ORP by MiOXSYS System	49 infertile men with palpable varicocele	**ORP:** Preoperative:4.73 mV/10^6 sperm/mL; 3 months postoperative: 2.03 mV/106 sperm/mL (P < 0.05 *versus* preoperative)

Cat :catalase activity ; DCFH-DA: 20, 70-dichlorodihydrofluorescein diacetate ; GPx: glutathione peroxidase activity; H2O2: hydrogen peroxide; IQR: interquartile range; MDA: malondialdehyde; NO: Nitric oxide; NS: not significant; ORP: oxidation-reduction potential; ROS: Reactive oxygen species; SA: semen analysis; SOD: superoxide dismutase activity; TAC: total antioxidant capacity;

pregnancy after MV and those who did not. Additionally, Tavalaee and colleagues used 20, 70-dichlorodihydrofluorescein diacetate (DCFH) to evaluate intracellular ROS levels and demonstrated that the mean percentages of DCFH-positive spermatozoa decreased postoperatively (from 47.6% to 36.6%; P=0.03) (136). Also, applying DCFH to evaluate OS, Barekat and colleagues reported an increase in the percentage of ROS-negative sperm (77.2% ± 7.5% vs. 92.3% ± 2.6%, P<0.05) 3 months after MV (137). Moreover, Abbasi and colleagues assessed sperm lipid peroxidation as an OS marker and described improvement after MV (36.22% vs. 24.04%; P=0.009) (138). Measuring the static oxidation-reduction potential (sORP) preoperatively and three months postoperatively, Kavoussi and colleagues found decreased sORP in infertile men with palpable varicocele who underwent MV (preoperative: 4.73 mV/106 sperm/ml vs. postoperative: 2.03 mV/106 sperm/ml, P<0.001) (139). Notably, the authors also described improved sperm DNA fragmentation levels after surgery; however, there was no correlation between sORP and sperm DNA damage.

Conversely, Mancini and colleagues, comparing TAC values between 25 infertile men with varicocele and 14 infertile men who had undergone MV 10-24 months before (140), did not find a difference between the two groups (106.6 ± 8.9 seconds vs. 105.8 ± 8.7 seconds). Moreover, while reporting a decrease in TAC from 2292 uM preoperatively to 1885 uM postoperatively (P=0.03), Baker and colleagues noticed that most participants persisted with TAC above the reference level (141). Additionally, the authors did not find a statistically significant difference in ROS or ROS-TAC scores after the procedure. The limited evidence points towards a beneficial effect of varicocelectomy in reducing OS in semen samples of infertile men.

Sperm DNA Fragmentation Levels After Varicocelectomy and Outcomes

Given the vital link between varicocele and sperm DNA fragmentation, the role of varicocele repair in improving sperm DNA has been under scrutiny (63). To date, four meta-analyses have been reported on this topic, and their findings will be summarized in this section (Table-4).

The first meta-analysis was published in 2012 by Wang and colleagues. The analysis included data from six studies involving 177 men with clinical varicocele (103). The authors reported a statistically significant reduction (weighted mean difference [WMD] of -3.4%; 95% CI: -4.1 to -2.5, P<0.05) in the sperm DNA fragmentation rates after varicocelectomy. However, these authors included one study of men using antioxidants (142) and another on adolescents (143). Additionally, they pooled the data irrespective of the type of assay used for sperm DNA fragmentation measurement.

In 2020, Qiu and colleagues performed a new meta-analysis including 394 participants from 11 studies and confirmed the findings of the previous study by Wang and colleagues. However, in their study, a larger effect size of varicocelectomy on sperm DNA fragmentation rates was found (WMD -5.79%; 95% CI -7.39 to -4.19, P<0.05) (144). The limitations of this study were the inclusion of one study with men who had varicocelectomy for reasons other than infertility (145), a study involving men with subclinical varicocele (110), another including adolescents (143), and a trial assessing sperm DNA fragmentation by the sperm chromatin protamination test (146), which is not optimal for detecting DNA strand breaks. Moreover, this study also pooled the data irrespective of the type of assay used for sperm DNA fragmentation measurement.

The meta-analysis by Birowo and colleagues, also published in 2020, analyzed seven prospective studies, including in total 289 infertile men with palpable varicocele, and found a reduction in sperm DNA fragmentation rates after varicocelectomy (WMD -6.9%; 95% CI -10.0% to -3.7%, P<0.05) (147). This study included few trials and participants and examined only the SCSA and TUNEL for sperm DNA fragmentation assessment. Moreover, a subanalysis by the type of sperm DNA fragmentation assay was not carried out.

In the most recent systematic review and meta-analysis, our group compiled data from 19 studies involving 1070 infertile men with palpable varicocele. In our study, varicocelectomy reduced postoperative sperm DNA fragmentation rates (all sperm DNA fragmentation assays combined; WMD -7.2%; 95% CI -8.9%; -5.6%; P<0.05) with a moderate effect size (Cohen's d=0.68;

Table 4. Characteristics of the meta-analyses assessing the effects of varicocelectomy on sperm DNA fragmentation

Author, year, (country)	Population	Type of Included Studies	SDF assay	Varicocelectomy technique	Number of studies and participants	Decrease %SDF after varicocelectomy	Limitations
Wang et al., 2012 (China) (103)	Infertile men with palpable varicocele and abnormal SA	Retrospective and prospective cohort	SCSA, TUNEL and Comet	Open non-microsurgical and open microsurgical	6 studies; 177 participants	WMD -3.37%; 95% CI: -4.09 to -2.65, P<0.05	One study included men using antioxidants, and another study included adolescents; Data were pooled irrespective of SDF assay type; Pregnancy and live birth rates not evaluated
Qiu et al., 2020 (China) (144)	Men with varicocele	Prospective cohort and case-control	SCSA, SCD, TUNEL, Comet, and AOT	Open non-microsurgical and open microsurgical	11 studies; 394 participants	WMD -5.79%; 95% CI -7.39 to -4.19, P<0.05	One study included fertile men, another included men with subclinical varicocele; one study included adolescents, and another trial assessed SDF by a sperm chromatin protamination test; data was pooled irrespective of SDF assay type; pregnancy and live birth rates not evaluated
Birowo et al., 2020 (Indonesia) (147)	Infertile men with palpable varicocele	Prospective cohort	SCSA and TUNEL	Open non-microsurgical and open microsurgical	7 studies; 289 participants	WMD -6.86%; 95% CI -10.04 to -3.69, P<0.05	Low number of studies and participants; data was pooled irrespective of SDF assay type; pregnancy and live birth rates not evaluated
Lira Neto et al., 2020 (Brazil) (23)	Infertile men with palpable varicocele	Retrospective and prospective cohort	SCSA, SCD, TUNEL and Comet	Open non-microsurgical, open microsurgical, and laparoscopic	19 studies; 1070 participants	WMD -7.23%; 95% CI -8.86; -5.59; P<0.05	Pregnancy and live birth rates not assessed

AOT: Acridine orange test; SDF: Sperm DNA Fragmentation; %SDF: sperm DNA fragmentation rate; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; SCSA: sperm chromatin structure assay; SCD: sperm chromatin dispersion test; WMD: Weight Mean Difference

95% CI: [WMD] 0.77-0.60) (23). When the studies were categorized by the type of sperm DNA fragmentation assay (TUNEL, SCSA, SCD, and alkaline Comet), the reduction in sperm DNA fragmentation levels remained significant, without major variation among assays. These findings align with studies demonstrating a moderate-to-high correlation between the assays used to measure sperm DNA fragmentation (94-96, 148). Furthermore, they corroborate recent data indicating a substantial intraindividual agreement in sperm DNA fragmentation rates evaluated in two ejaculates from the same subjects within a 3-month interval (149).

In the study mentioned above, we have also evaluated the influence of the surgical technique on the improvement of sperm DNA fragmentation and found a similar effect size for microsurgical (WMD -7.2%, 95% CI -8.9%, -5.4%; P<0.05) and open non-microsurgical techniques (WMD -7.1%, 95% CI -12.7%, -1.5%; P<0.05). Corroborating this finding, a comparative review of different approaches for varicocele repair revealed that open techniques, mainly micro-

surgery, yielded more significant improvements in semen parameters and pregnancy rates than other techniques (123).

Furthermore, in subanalysis by baseline sperm DNA fragmentation levels, we demonstrated that men with preoperative levels >20% had a more significant reduction of sperm DNA fragmentation compared to those with levels <20% (all sperm DNA fragmentation assays combined; WMD -8.3% vs. -3.9%, P<0.05). Furthermore, we conducted a meta-regression analysis revealing that sperm DNA fragmentation improved postoperatively as a function of preoperative sperm DNA fragmentation levels (Coefficient: 0.23; 95% CI: 0.07-0.39; P<0.05) (Figure-3). These findings suggest that men with high sperm DNA fragmentation levels at baseline benefit the most from varicocele repair, similar to the recommendations of varicocelectomy regarding basic semen analysis parameters (12).

Concerning the improvement of sperm DNA fragmentation to levels lower than the threshold of 30%, Werthman and colleagues studied 11 infertile men with palpable varicocele, abnormal basic SA, and baseline SDF>30%. The authors reported that 64% of the participants reached SDF levels <30% 3 to 6 months after varicocelectomy (150). Similarly, Ghazi and colleagues found that 88% of men with preoperative sperm DNA fragmentation >30% improved to levels <30% following MV (151).

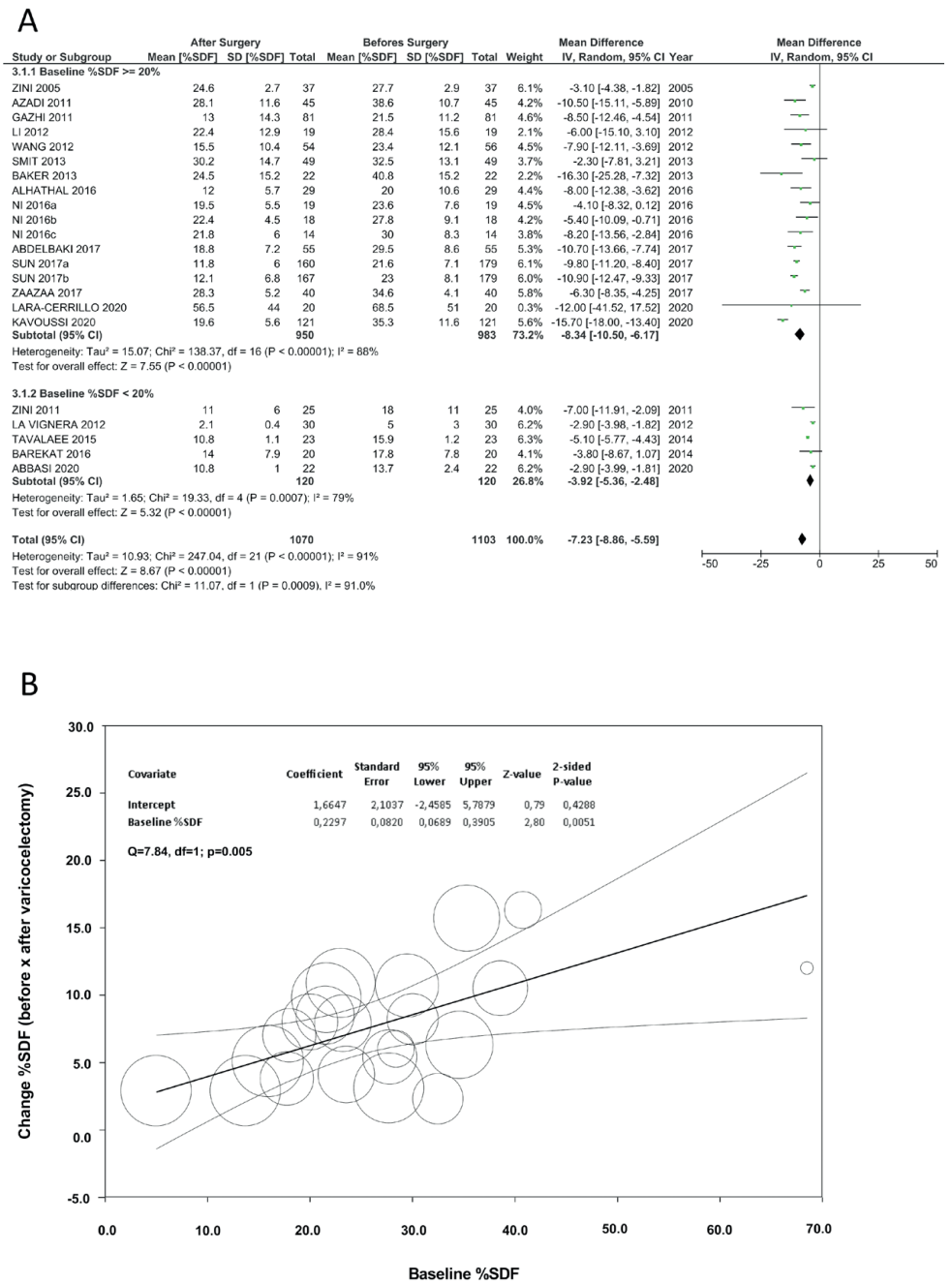
The influence of varicocele grade on the outcomes of varicocelectomy has been highlighted by a recent meta-analysis that showed a greater improvement in basic SA parameters in men with grade 2 and 3 varicocele (114). Despite the association between sperm DNA fragmentation improvement in all varicocele grades mentioned in our study (23), we could not perform a subanalysis by grade due to the small number of studies that provided such data (61, 152-154).

Subclinical Varicocele. Only two studies have investigated the effect of repairing subclinical varicoceles on sperm DNA fragmentation levels. The study by Garcia-Peiró and colleagues included infertile men with subclinical varicocele diagnosed by scrotal Doppler ultrasonography and found no difference in the sperm DNA fragmentation levels between the participants who underwent varicocelectomy and those who did not (31.4% vs. 28.9%, by TUNEL) (110). Furthermore, employing SCSA to measure sperm DNA fragmentation, Sun and colleagues evaluated 358 infertile men with left clinical and right subclinical varicocele, randomized to undergo bilateral (n = 179) or unilateral (n = 179) microsurgical subinguinal varicocelectomy (155). The authors reported more significant improvement in basic semen analysis parameters and higher natural pregnancy rates in the bilateral varicocele repair group compared with the unilateral varicocele group. However, sperm DNA fragmentation levels were not statistically different among the groups both preoperatively (21.6% ± 7.1% vs. 23.0% ± 8.1%) and postoperatively (11.8% ± 6.0% vs. 12.1% ± 6.8%) (155).

The timing for sperm DNA fragmentation retesting after varicocelectomy has also been studied. Most authors recommend a follow-up test between 3 to 6 months after the procedure, similar to the recommendation regarding basic SA parameters. This suggestion is based on the duration of spermatogenesis in humans, which is approximately 72 days (11). Thus, waiting more than 90 days ensures that at least one wave of spermatogenesis has progressed under the procedure's benefit. Some studies have demonstrated a progressive decline in sperm DNA fragmentation levels with increasing follow-up time after varicocelectomy (105). In contrast, others found consistently lower sperm DNA fragmentation levels in the postoperative period (e.g., three months), without further significant improvement over time (61, 156).

The association between the improvement of sperm DNA fragmentation and reproductive outcomes has been the objective of few studies. Smit and colleagues, studying infertile men with palpable varicocele and oligozoospermia, found lower postoperative sperm DNA fragmentation levels in couples that conceived naturally or with ART exhibited compared to those who did not (26.6% ± 13.7% vs. 37.3% ± 13.9%, P<0.05) (157). Similarly, Ni and colleagues demonstrated that infertile men with palpable varicocele and abnormal semen analysis who achieved pregnancy naturally six months after varicocelectomy had decreased sperm DNA fragmentation rates compared to preoperative values (17.6% ± 3.4% vs.

Figure 3. Varicocele Repair on Sperm DNA Fragmentation.



(A) Forest plot showing the Weighted Mean Difference (WMD) for sperm DNA fragmentation (SDF) rates after varicocelectomy (versus before) according to baseline (preoperative) SDF levels. CI = confidence interval; IV = inverse variance; (B) Meta-regression showing the influence of baseline SDF levels (moderator) on %SDF change after varicocelectomy. The size of the circles indicates each study's sample size. The solid line represents linear predictions for the %SDF change after varicocelectomy as a function of the mean absolute increase in preoperative SDF levels. The curved lines indicate the 95% confidence interval around the regression line (42, 61, 136-138, 141, 151-157, 169, 170, 172-175). Reprinted with permission from Elsevier; Lira Neto et al. Effect of varicocelectomy on sperm deoxyribonucleic acid fragmentation rates in infertile men with clinical varicocele: a systematic review and meta-analysis. Fertil Steril. 2021 Sep;116(3):696-712.

26.8% ± 8.6%, P<0.05) and non-pregnant patients (17.6 ± 3.4% vs. 22.3 ± 5.4%; P<0.05) (61). Likewise, Wang and colleagues found that the mean postoperative sperm DNA fragmentation rate in infertile men with clinical varicocele and elevated sperm DNA fragmentation levels who underwent varicocele repair and fathered a child was lower than in those who did not conceive (13.9% ± 9.7% vs. 20.1% ± 10.3%, P<0.05), (152). Furthermore, in a prospective study including 75 infertile men with palpable varicocele and abnormal SA parameters, Mohammed and colleagues reported that couples that achieved natural pregnancy at 1-year follow-up after the procedure had significantly lower sperm DNA fragmentation levels than those who did not (16.4% ± 6.4% vs.24.2 ± 4.1%, P<0.05) (158). In contrast, in a retrospective study including 24 infertile men with palpable varicocele, no difference in sperm DNA fragmentation levels was found between pregnant and non-pregnant couples after MV (22.2% ± 14.4% vs. 25.7% ± 14.5%, P=0.6), despite a significant decrease in the mean sperm DNA fragmentation rates after the operation (preoperative: 40.8% vs postoperative: 24.5%; P<0.05) (141).

The studies summarized above indicate that varicocele repair in infertile men with palpable varicocele reduces sperm DNA fragmentation levels. Furthermore, sperm DNA integrity improvement after the intervention seems to enhance the chance of natural conception. However, data substantiating the latter statement is still limited and overwhelmingly based on observational studies.

Practice change: Updated Varicocelectomy Indications

In most practice guidelines from professional societies, varicocele repair is only recommended for infertile men with palpable varicocele and abnormal basic semen parameters (i.e., sperm concentration, sperm motility, or sperm morphology) (12). However, basic semen analysis parameters lack accuracy in assessing the male fertility potential (13, 84-86, 159). Moreover, recent evidence referenced in this review highlights the critical relationship among varicocele, OS, and sperm DNA fragmentation, as well as their negative effect on male fertility. Therefore, this topic has been revisited recently, and new guidelines have already suggested that elevated sperm DNA fragmentation levels should be considered an indication of varicocelectomy repair in infertile males with palpable varicocele, even for patients with basic SA parameters within the WHO normal ranges.

For instance, the latest European Urology Association (EAU) male infertility guideline includes a strong recommendation for sperm DNA fragmentation assessment in men with otherwise unexplained infertility or who have reported failed ART, including recurrent pregnancy loss or failure of embryo development and implantation (116). The same document goes further, including a weak recommendation for varicocelectomy in men with elevated sperm DNA fragmentation levels in the same scenarios (i.e., unexplained infertility, recurrent pregnancy loss, failure of embryo development or implantation). In addition, the guidelines highlight that OS has been recognized as a cause of male infertility, albeit stating that seminal ROS testing cannot be recommended in routine clinical practice until its diagnostic utility is validated by further studies (116).

The Sperm DNA Fragmentation Study Group (SFRAG) issued a guideline summarizing the evidence concerning the impact of sperm DNA fragmentation in different clinical settings, such as varicocele, unexplained infertility, idiopathic infertility, recurrent pregnancy loss, intrauterine insemination, in vitro fertilization/intracytoplasmic sperm injection, fertility counseling for men with infertility risk factors, and sperm cryopreservation (24). Regarding varicocele, the SFRAG guideline states that assessing sperm DNA fragmentation levels in infertile men is helpful when deciding about treatment options, especially in men with low-grade varicocele or in those with normal or borderline basic SA parameters. Furthermore, it highlights that determining postoperative sperm DNA fragmentation levels helps guide further treatments (24). The SFRAG guideline also provides helpful recommendations for the decision-making process when dealing with infertile men with varicocele, even in cases where varicocelectomy is not warranted by itself according to the traditional indication, i.e., when basic semen parameters are within the reference ranges. For instance, it states that sperm DNA fragmentation testing might also be helpful for infertile men with pal-

pable varicocele who are candidates for ART. In these cases, varicocelectomy could be considered before ART for patients with elevated sperm DNA fragmentation to improve reproductive outcomes (160). Importantly, as reasoned by researchers in recent articles, only through a comprehensive andrological evaluation, including a detailed medical and reproductive history and physical examination, and additional investigations such as sperm DNA fragmentation testing, will correctable conditions such as varicocele be detected and optimally treated, allowing the couple to achieve the best reproductive outcomes possible potentially (13, 84, 161).

Knowledge Gaps and Future Research

Current literature indicates a strong link between varicocele-related infertility and OS. However, further data is needed from prospective studies, including fertile controls and fertile and infertile men with varicocele, with large sample sizes from various institutions and countries, to better describe the prevalence and natural history of OS among men with varicocele. In addition, standardization of the methods used for OS assessment, as well as subanalyses by varicocele grade and laterality, should be performed in these studies.

Regarding the impact of varicocelectomy on seminal ROS levels, there is level 2 evidence indicating an improvement of OS markers in infertile men with varicocele. However, due to the small number of studies and the lack of standardization of the different methods used to assess OS, further prospective studies with larger sample sizes and simultaneously applying direct and indirect techniques to measure ROS are needed to produce stronger evidence. Additionally, subanalyses by varicocele grade, laterality, type of ROS assay, and baseline ROS levels should be carried out. More importantly, the relationship between the improvement of OS after varicocele repair and reproductive outcomes must be evaluated.

Level 1 evidence concerning the negative association between varicocele and sperm DNA fragmentation and the positive effect of varicocele repair on sperm chromatin integrity already exists. Nevertheless, some points remain to be elucidated. The exact prevalence and natural history of elevated sperm DNA fragmentation among varicocele patients are still unclear. Similarly, data concerning the influence of varicocele grade and laterality on preoperative and postoperative sperm DNA fragmentation levels is limited. Thus, larger and multicentric cohort studies and clinical trials with subgroup analyses by varicocele grade and laterality are needed.

Future research is also warranted to clarify whether varicocelectomy can also improve sperm DNA fragmentation in men with basic SA parameters within the WHO reference ranges, as well as the proportion of patients with high baseline sperm DNA fragmentation levels that reach normal levels after varicocelectomy (162). Clinical trials including this population should be performed, ideally including a group of infertile men with varicocele, abnormal basic SA parameters, and high sperm DNA fragmentation to compare outcomes. Moreover, further studies should assess sperm DNA fragmentation levels at different time intervals after varicocele repair and their relationship with pregnancy outcomes in both natural and ART scenarios. Clinical trials with serial postoperative measurements of sperm DNA fragmentation and a follow-up of at least 12 months are needed. Preoperative sperm DNA fragmentation levels may also be included in nomograms created to predict the reproductive outcomes of varicocele repair at the individual level.

Lastly, there are knowledge gaps concerning the specific pathways by which varicocele causes OS and sperm DNA fragmentation and how varicocelectomy improves sperm chromatin integrity and decreases ROS production. Studies using 'omics' techniques may illuminate the relevant metabolic pathways (48, 163, 164). Table-5 summarizes the main knowledge gaps and the further research needed, as discussed above.

CONCLUSIONS

A growing evidence body supports oxidative stress and sperm DNA damage as critical factors in the pathophysiology of varicocele-related infertility. However, the pathways by which varicocele causes oxidative stress are not fully understood. In some men with varicocele, defense mechanisms against excessive ROS production are defective, leading to spermatogenesis impairment and subsequent infertility. Sperm DNA frag-

Table 5. Main knowledge gaps regarding varicocele, oxidative stress, and sperm DNA fragmentation

Knowledge gaps	Suggested studies
Mechanisms by which varicocele causes OS.	Proteomics and Metabolomics studies in men with and without varicocele.
Impact of varicocele laterality on OS.	Cross-sectional studies in infertile men with unilateral *versus* bilateral varicocele.
Impact of subclinical varicocele on OS.	Cross-sectional studies in infertile men with subclinical *versus* palpable varicocele.
Definition of cut-off levels for the different OS markers for men with varicocele	Cross-sectional studies including healthy fertile normozoospermic men (controls), fertile men with varicocele, and infertile men with varicocele, using several markers of OS (Total ROS, MDA, 8-OHdG, and TAC) simultaneously, providing ROC curve analysis for each marker.
Impact of time on varicocele-induced OS.	Prospective cohort studies with fertile and infertile men with varicocele measuring OS markers in serial time points.
Impact of varicocelectomy on varicocele-induced OS	Prospective cohort studies including infertile men measuring various OS markers before and after varicocele repair, including subanalyses by varicocele grade, laterality, baseline OS marker levels, and surgical technique. Measuring various OS markers in serial time points is also recommended.
Impact of time on varicocele-induced sperm DNA fragmentation.	Prospective cohort studies with fertile and infertile men measuring sperm DNA fragmentation in serial time points.
Proportion of men with varicocele and increased sperm DNA fragmentation levels	Cross-sectional studies including healthy, fertile normozoospermic men (controls), fertile men with varicocele, and infertile men with varicocele. Cut-off levels should be defined for each assay based on the literature.
Impact of varicocele grade on sperm DNA fragmentation.	Cross-sectional studies in infertile men grouped by varicocele grade (including subclinical).
Impact of varicocele laterality on sperm DNA fragmentation.	Case-control studies in infertile men with unilateral *versus* bilateral varicocele.
Impact of varicocelectomy on varicocele-induced sperm DNA fragmentation	Prospective cohort studies including infertile men with increased sperm DNA fragmentation levels undergoing varicocele repair. Subanalyses by varicocele grade, laterality, and surgical technique should be performed. Measuring sperm DNA fragmentation in serial time points is also recommended.
Association between OS and sperm DNA fragmentation levels in men with varicocele	Cross-sectional studies including healthy fertile normozoospermic men (controls), fertile men with varicocele, and infertile men with varicocele, and measuring simultaneously several OS markers as well as sperm DNA fragmentation
Association between the improvement of OS and sperm DNA fragmentation levels after varicocele repair.	Prospective cohort studies including infertile men with increased OS markers and sperm DNA fragmentation levels undergoing varicocele repair. Several OS markers and sperm DNA fragmentation should be measured simultaneously and in serial time points.
Association between the improvement of OS and sperm DNA fragmentation levels after varicocele repair with natural pregnancy outcomes.	Prospective cohort studies including infertile men with increased OS markers and sperm DNA fragmentation levels undergoing varicocele repair. Participants should be followed up for at least 12 months after surgery in couples actively trying to conceive.
Association between the improvement of OS and sperm DNA fragmentation levels after varicocele repair with ART outcomes.	Prospective cohort studies including infertile men with increased OS markers and sperm DNA fragmentation levels undergoing varicocele repair. Participants should wait at least 3 months after surgery for ART treatments and should be followed up until the end of each treatment.

8-OHdG: 8-hydroxy-2'-deoxyguanosine; ART: assisted reproduction techniques; MDA: malondialdehyde; OS: Oxidative stress; ROS: Reactive oxygen species; SA: semen analysis; TAC: total antioxidant capacity;

mentation is one of the adverse effects of varicocele-induced oxidative stress; elevated sperm DNA fragmentation levels decrease the chance of natural conception and ART success. Varicocele repair may restore the balance between reactive oxygen species and antioxidants, alleviating sperm DNA damage and improving the likelihood of natural and assisted pregnancy in men with palpable varicocele and infertility. These findings have resulted in changes to clinical practice guidelines, incorporating sperm DNA fragmentation testing for infertile men with palpable varicocele and varicocelectomy in cases of elevated sperm DNA fragmentation levels. Gaps in knowledge exist, including understanding the mechanisms behind increased ROS production and sperm DNA fragmentation in men with varicocele. In addition, the impact of varicocele grade and laterality on OS and sperm DNA fragmentation, as well as the effect of improved OS and sperm DNA fragmentation levels in pregnancy and live birth rates after varicocelectomy, are still unclear and deserve further investigation.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Sandro C. Esteves, MD, PhD
ANDROFERT - Clínica de Andrologia e Reprodução Humana, Centro de Ref. em Reprodução Masculina
Av. Dr. Heitor Penteado, 1464
Campinas, Brasil
E-mail: s.esteves@androfert.com.br

ORIGINAL ARTICLE

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High-risk patients for septic shock after percutaneous nephrolithotomy

Alexandre Danilovic [1], Lucas Piraciaba Cassiano Dias [1], Fabio Cesar Miranda Torricelli [1], Giovanni Scala Marchini [1], Carlos Batagello [1], Fabio Carvalho Vicentini [1], William C. Nahas [2], Eduardo Mazzucchi [1]

[1] Departamento de Urologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil; [2] Disciplina de Urologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil

ABSTRACT

Purpose: to identify risk factors for urinary septic shock in patients who underwent percutaneous nephrolithotomy (PCNL).

Materials and Methods: Data from PCNL procedures performed between January 2009 and February 2020 were retrospectively analyzed. The study included all patients over 18 years old with kidney stones larger than 15 mm who underwent PCNL. Patients who underwent mini-PCNL or combined surgeries, such as ureteroscopy or bilateral procedures, were not included in the study. Logistic regression was conducted to determine the risk factors for urinary septic shock within 30 days post-operation in patients who underwent PCNL.

Results: Urinary septic shock was observed in 8 out of the 1,424 patients analyzed (0.56%). The presence of comorbidities, evaluated using the Charlson Comorbidity Index (CCI) (OR 1.46 [CI 95% 1.15-1.86], p=0.01), larger stones (41.0 mm [IQR 30.0-47.5 mm] vs. 24.0 mm [IQR 17.0-35.0 mm], OR 1.03 [CI 95% 1.01-1.06], p=0.04), and a positive preoperative urine culture (OR 8.53 [CI 95% 1.71-42.45], p <0.01) were shown to significantly increase the risk of postoperative urinary septic shock. Patients with a CCI > 2, larger stones (≥ 35 mm), and a positive preoperative urine culture were at even higher risk of urinary septic shock (OR 15.40 [CI 95% 1.77-134.21], p=0.01).

Conclusion: Patients with larger stones, positive preoperative urine culture, and a higher CCI are at risk for urinary septic shock after PCNL. These findings are of utmost importance for optimizing the perioperative care of these patients to prevent life-threatening complications.

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Alexandre Danilovic
https://orcid.org/0000-0002-6963-6117

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INTRODUCTION

Percutaneous nephrolithotomy (PCNL) is the first line therapy for large kidney stones (1). Despite the reduction in complications associated with PCNL due to technological improvements, postoperative infectious complications remain a significant challenge (2-4). Approximately 10% of the patients experience transient postoperative fever, while 0.3-0.5% develop urinary sepsis after PCNL (5, 6). Although rare, sepsis is a dramatic complication and constitutes the primary cause of mortality among patients undergoing PCNL (7).

The literature lacks a clear consensus on defining high-risk patients for postoperative infectious complications of PCNL. Most previous studies use postoperative fever as their primary endpoint, and the changing definitions of the terms "sepsis" and "septic shock" in recent years have led to different outcomes (8). A prospective study demonstrated that postoperative fever is associated with *diabetes mellitus*, positive urine culture (particularly Gram-negative pathogen), staghorn calculus, and the preoperative use of a nephrostomy tube (6). Other authors have demonstrated that the need for blood transfusion, larger stone size and prolonged operative time were risk factors for post-PCNL sepsis. Possible predictors, such as positioning in PCNL, tract size, obesity, and a solitary kidney did not impact infectious rates in most of the previous studies (9-13). As a result, there are no specific recommendations for antibiotic prophylaxis due to a lack of sufficient data (14-16). A recent meta-analysis demonstrated a decrease in the risk of sepsis in the postoperative period of PCNL with the use of oral antibiotic prophylaxis for one week before surgery, regardless of the patient risk factors (15). In order to optimize the use of perioperative antibiotics for PCNL, we need more studies to better understand the risk factors for severe infectious complications of PCNL such as septic shock. Currently, EAU Guidelines states that the available evidence for prevention of infection following percutaneous stone removal remains limited (1).

Our hypothesis is that identifying high infectious risk patients for PCNL would facilitate the establishment of specific recommendations for antibiotic prophylaxis and the reduction of infectious complications in these patients. The primary objective of this study is to evaluate predictors associated with the progression to postoperative PCNL sepsis shock resulting from urinary tract infection (UTI).

MATERIALS AND METHODS

Data from PCNL procedures performed between January 2009 and February 2020 at a single specialized center were retrospectively analyzed. The study included all patients over 18 years old with kidney stones larger than 15 mm who underwent PCNL. We did not restrict the inclusion of multiple events for a single patient. Patients who underwent mini-PCNL or combined surgeries, such as ureteroscopy or bilateral procedures, were not included in the study. The institutional ethics committee approved this study (IRB approval number 75906623.0.0000.0068).

Personal clinical features, such as age, gender, body mass index (BMI), and comorbidities, were evaluated. These factors were analyzed using the Charlson Comorbidity Score (CCI) and the *American Society of Anesthesiologists* score (ASA) (17, 18). Preoperative urinary drainage using a ureteral catheter or a nephrostomy tube and whether the event was indicated as a second-look surgery were also evaluated. Radiological characteristics of the stones and the urinary tract were assessed by preoperative non-contrast computed tomography (NCCT) (19). These features included the largest diameter of the addressed stones, the presence of hydronephrosis, and the analysis of the *Guy's Stone Score* (20).

Patients underwent PCNL in either the prone position or one of the supine position variations (complete supine, Galdakao modified Valdivia, or Barts flank-free position), which are commonly employed at our Institution, selected according to the surgeon's preference. All patients received a third-generation cephalosporin during the induction of anesthesia, except for those with a positive urine culture who received antibiotics guided by the urine culture results. A negative urine culture was obtained before the procedure. Following the general anesthesia, we initiated the procedure with a cystos-

copy to place a 6 French (Fr) ureteral catheter up to the side of the addressed stone. After proper positioning, the calyceal punctures were guided by a retrograde pyelogram, ultrasound, or a combination of both techniques. The procedure was interrupted if pyuria was observed by the surgeon, and a nephrostomy tube was then inserted. Subsequently, the urinary tract was dilated using plastic dilators (Amplatz dilators), and nephroscopy was performed using a 26-F nephroscope (Karl Storz, Tuttlingen, Germany). Stone fragmentation was achieved using an ultrasonic lithotripter (Swiss Lithoclast Master, EMS, Switzerland). At the conclusion of the procedure, either a ureteral stent or a nephrostomy tube was placed based on the surgeon's preference. Stone-free status was routinely assessed by NCCT performed on the first postoperative day (POD) (21, 22).

Additionally, we conducted a microbiological analysis, which involved a urine culture collected on the day before the procedure, an analysis of the composition of the calculus, and the culture of the calculus itself. Regarding perioperative data, we analyzed the operative time and hematimetric variation.

We employed a dual-check approach to define sepsis. We combined the diagnosis provided during the event with a retrospective diagnosis based on the updated Sepsis-3 criteria: sepsis as organ dysfunction caused by the response to infection, utilizing the diagnosis of infection and a *Sequential Organ Failure Assessment (SOFA)* score ≥ 2. Septic shock definition was a circulatory dysfunction identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg and serum lactate level >2 mmol/L (>18 mg/dL) (23). Additionally, we checked the data of all patients who required intensive care in the postoperative period following PCNL.

Statistical Analysis

Measures of central tendency and dispersion, as well as absolute and relative frequencies, were calculated for participant characterization and the analysis of clinical and sociodemographic characteristics of participants, stratified by the presence or absence of urinary septic shock.

Due to the dichotomous nature of the dependent variable, we employed logistic regression for bivariate analyses. We adopted a significance level of $\alpha < 0.05$ and a 95% confidence interval (CI 95%). The Stata/SE software version 12 (StataCorp LLC®, United States) was utilized for data analysis.

RESULTS

Among the analysis of the 1,424 surgical procedures, we observed a predominance of women (63.3%), with a median age of 47 years and a BMI of 27.8 kg/m^2 (Table-1). The primary outcome of postoperative urinary septic shock occurred in 8 of the 1,424 patients (0.56%). We recorded two deaths due to septic shock, resulting in a lethality rate of 0.14% in our study. One patient died on the 7 POD and the other on the 12 POD due to organic dysfunction caused by urinary sepsis.

Hemorrhagic shock was the cause of admission to the intensive care unit (ICU) in eight patients, with two deaths in this group. An additional eight patients were admitted to the ICU for various reasons, including renal insufficiency, iatrogenic colon injury, drug-induced hepatitis, adrenal insufficiency, and acute cardiac decompensation, resulting in one death due to acute myocardial infarction.

The presence of comorbidities, as assessed by the CCI, demonstrated a significant risk of postoperative urinary septic shock (OR 1.46 [CI 95% 1.15-1.86], p=0.01). Patients who developed urinary septic shock had a median CCI score of 2 [IQR 0-5] vs. 1 [IQR 0-2] (Table-2). Larger stones also showed a higher risk of progressing to urinary septic shock (OR 1.03 [CI 95% 1.01-1.06], p=0.04). The median stone size for patients who developed urinary septic shock was 41.0 mm [IQR 30.0-47.5 mm] vs. 24.0 mm [IQR 17.0-35.0 mm] in other patients. We used the highest interquartile range of the patients who did not develop urinary septic shock to analyze stone size as categorical data. Stones ≥ 35.0 mm demonstrated a higher risk of progressing to urinary septic shock when compared to smaller stones (OR 4.51 [CI 95% 1.08-18.94], p=0.04). A positive preoperative urine culture was associated with a higher chance of urinary septic shock (OR 8.53 [CI 95% 1.71-42.45], p <0.01). Patients with CCI

Table 1 - Clinical features of 1424 patients included in the study.

Features	(n) (%) or Median [IQR]
Female	903 (63.4)
Age	47.0 [36.0-57.0]
BMI	27.8 [24.4-31.9]
ASA score	
1	459 (32.3)
2	836 (58.8)
3	126 (8.8)
4	2 (0.1)
Charlson Comorbidity Index	
0	664 (46.7)
1	336 (23.6)
2	220 (15.5)
3	122 (8.6)
4	43 (3.0)
5	18 (1.3)
6	12 (0.8)
7	3 (0.2)
8	1 (0.1)
9	1 (0.1)
10	2 (0.1)
13	1 (0.1)
Stone diameter (mm)	24.0 [17.0-35.0]
Guys's score	
1	67 (4.7)
2	426 (30.0)
3	657 (46.3)
4	268 (18.9)
Hydronephrosis	906 (64.8)
Preoperative ureteral stent	287 (20.2)
Preoperative urinary drainage	314 (22.1)
Second-look surgery	96 (6.8)

Urine culture	
Positive	368 (26.1)
Escherichia coli	138 (9.8)
Proteus mirabilis	55 (3.9)
Group B Streptococcus	36 (2.6)
Coagulase-negative Staphylococcus	30 (2.1)
Klebsiella pneumoniae	28 (2.0)
Pseudomonas aeruginosa	16 (1.1)
Staphylococcus aureus	10 (0.7)
Other bacteria and fungi	55 (3.9)
Negative	1039 (73.9)
Stone culture	
Positive	300 (36.1)
Coagulase-negative Staphylococcus	79 (9.5)
Escherichia coli	66 (8.0)
Proteus spp	39 (4.7)
Enterococcus spp	29 (3.5)
Klebsiella pneumoniae	17 (2.0)
Pseudomonas spp	17 (2.0)
Staphylococcus aureus	10 (1.2)
Streptococcus viridans	8 (1.0)
Other bacteria and fungi	35 (4.2)
Negative	530 (63.9)
Stone composition	
Struvite	630 (50.6)
Calcium oxalate	273 (21.9)
Mixed calcium stones (oxalate, phosphate, carbonate, uric acid)	205 (16.4)
Calcium oxalate + calcium phosphate	83 (6.7)
Uric acid	41 (3.3)
Cystine	14 (1.1)
Stone free procedures	684 (48.3)
Operative time, minutes	120 [90-160]
Hematocrit drop (%)	4.3 [1.9 -7.2]

IQR = interquartile range; BMI = Body Mass Index; ASA = American Society of Anesthesiologists; mm = milimeter

Table 2 - Analysis of predictors for urinary septic shock after PCNL.

Features	Total (N=1,424) (%)	Urinary septic shock (N=8) (%)	No urinary septic shock (N=1,416) (%)	p value	*Odds ratio [CI 95%]**
Gender					
Female	903 (63.4)	5 (62.5)	898 (63.4)	0.96	
Age	47.0 [36.0-57.0]	53.0 [36.5-65.5]	47.0 [36.0-57.0]	0.22	
BMI	27.8 [24.4-31.9]	26.4 [23.4-28.5]	27.8 [24.4-32.0]	0.34	
ASA score					
1	459 (32.3)	2 (25.0)	457 (32.3)	0.08	
2	836 (58.8)	3 (37.5)	833 (58.9)		
3	126 (8.8)	3 (37.5)	123 (8.7)		
4	2 (0.1)	0 (0.0)	2 (0.1)		
CCI	1 [0-2]	2 [0-5]	1 [0-2]	0.01	1.46 [1.15-1.86]
Diabetes	136 (9.6)	2 (25.0)	134 (9.5)	0.20	
Stone diameter (mm)	24.0 [17.0-35.0]	41.0 [30.0-47.5]	24.0 [17.0-35.0]	0.04	1.03 [1.01-1.06]
Stone diameter (mm)					
< 35mm	1032 (72.8)	3 (37.5)	1029 (73.0)	0.04	4.50 [1.08-18.93]
≥ 35mm	386 (27.2)	5 (62.5)	381 (27.0)		
Guy's score					
1	67 (4.7)	0 (0.0)	67 (4.7)	0.90	
2	426 (30.0)	2 (25.0)	424 (30.1)		
3	657 (46.3)	4 (50.0)	653 (46.3)		
4	268 (18.9)	2 (25.0)	266 (18.9)		
Hydronephrosis	906 (64.8)	6 (75.0)	900 (64.7)	0.53	
Previous urinary drainage	314 (22.1)	3 (37.5)	311 (22.0)	0.32	
Second-look surgery	101 (7.1)	0 (0.0)	101 (7.1)	---	
Urine culture					
Positive	370 (26.3)	6 (75.0)	364 (26.0)	<0.01	8.53 [1.71-42.45]
E. coli	138 (9.8)	5 (62.5)	133 (9.5)	<0.01	19.45 [3.73-101.27]
Other bacteria	232 (16.5)	1 (12.5)	231 (16.5)		2.24 [0.20-24.81]

Negative	1037 (73.7)	2 (25.0)	1035 (74.0)		1
Positive urine culture + Stone ≥ 35 mm	116 (8.3)	4 (50.0)	112 (8.0)	<0.01	11.46 [2.83-46.42]
CCI > 2 + Positive urine culture + Stone ≥ 35 mm	14 (1.0)	1 (12.5)	13 (0.9)	0.01	15.40 [1.77-134.21]
Stone culture					
E. coli	66 (7.9)	2 (33.3)	64 (7.8)	0.36	
Other bacteria	234 (28.2)	0 (0.0)	234 (28.4)		
Negative	530 (63.9)	4 (66.7)	526 (63.8)		
Stone composition					
Struvite	630 (50.6)	5 (62.5)	625 (50.5)	0.32	
Calcium oxalate	273 (21.9)	1 (12.5)	272 (22.0)		
Mixed calcium stones (oxalate, phosphate, carbonate, uric acid)	205 (16.4)	0 (0.0)	205 (16.6)		
Calcium oxalate + calcium phosphate	83 (6.7)	1 (12.5)	82 (6.6)		
Uric acid	41 (3.3)	1 (12.5)	40 (3.2)		
Cystine	14 (1.1)	0 (0.0)	14 (1.1)		
Stone free rate	684 (48.3)	3 (37.5)	681 (48.4)	0.54	
Operative time, minutes	120 [90-160]	125 [120-145]	120 [90-160]	0.99	
Prone position	675 (47.4)	6 (75.0)	669 (47.3)	0.12	
Hematocrit drop (%)	4.3 [1.9 -7.2]	5.5 [2.5-6.5]	4.3 [1.9 -7.2]	0.91	

* Calculated only for those that showed statistical significance.

BMI = Body Mass Index; ASA = American Society of Anestesiologists; CCI = Charlson Comorbidity Index; mm = milimeter

> 2, larger stones (≥ 35 mm), and a positive preoperative urine culture were at even higher risk of urinary septic shock (OR 15.40 [CI 95% 1.77-134.21], p=0.01).

Escherichia coli was the most frequent agent causing urinary septic shock (OR 19.45 CI [3.73-101.27], p <0.01), as it was present in five out of eight patients who experienced the primary outcome. Among the other three individuals, we observed one patient with a preoperative urine culture isolating *K. pneumoniae* and two patients with negative urine cultures, both of whom had a history of obstructive pyelonephritis.

The culture of the stones tested positive in 300 individuals (36.0%, n=830). Coagulase-negative *Staphylococcus* was isolated in 79 stone cultures (26.3% of the agents) and *E. coli* in 66 cases (22.0%). Among other isolated bacteria, there were 39 cases of *Proteus spp*,

29 cases of *Enterococcus spp*, 17 cases of *Klebsiella pneumoniae*, 17 cases of *Pseudomonas spp*, 10 cases of *Staphylococcus aureus*, and eight cases of *Streptococcus viridans.* Two individuals with positive cultures of the stone for E. coli developed urinary septic shock in the postoperative period, constituting one-third of patients from whom calculus culture was obtained, as is the only agent isolated in this group of patients, as depicted in Table-2.

A total of 1,246 events were evaluated with an analysis of calculus composition, resulting in 630 struvite stones (50.6%), 273 calcium oxalate stones (21.9%), 41 uric acid calculi (3.3%), 38 calcium oxalate and phosphate calculi (6.17%), 20 cystine calculi (1.1%), and the remaining composed of mixed stones containing calcium oxalate, calcium phosphate, calcium carbonate and uric acid.

DISCUSSION

This study demonstrates that a higher CCI, larger renal stones, and a positive preoperative urine culture are risk factors for urinary septic shock in patients undergoing PCNL. Furthermore, for the first time we demonstrated a 15-fold increase in the risk of urinary septic shock with the association between these factors, regardless of operative time or patient position during PCNL. Preoperative knowledge of these new findings may assist in identifying high-infectious risk patients for this life-threatening complication to optimize perioperative management for such individuals.

Our study has shown that a higher CCI is a risk factor for developing urinary septic shock. Our results confirm the greater vulnerability following the manipulation of stones in these patients, which may be associated with their chronic state of immunosuppression. Gutierrez *et al.* also studied the relationship between comorbidities and post-PNCL infectious complications, demonstrating that *diabetes mellitus* was a risk factor for postoperative fever (OR = 1.38, [CI 95% 1.05-1.81]) (6). However, it is important to distinguish the differences between the analyzed endpoints. Most postoperative fevers are transient and benign situations in which patients do not develop the life-threatening condition of septic shock. Additionally, there are some risks of bias in the postoperative fever outcome due to the routine use of antipyretic drugs in the postoperative period and the different temperature thresholds used to define fever among distinct studies.

Larger kidney stones presented a greater risk of septic shock in our study. Large-sized stones may be associated with the increased release of endotoxins and bacteria into the bloodstream (24, 25). This observation may be connected to the formation of infectious calculi. These stones tend to be larger, demanding procedures that last longer. However, our study failed to demonstrate that prolonged operative time was a risk factor for urinary septic shock. Wang et al., in a retrospective study involving 420 patients, found that operative time > 90 min was a significant risk factor for septic shock (26). Recently, Bansal et al. demonstrated in their retrospective study (N=580) that, in addition to prolonged operative time and the need for blood transfusions, the presence of stones >25mm was a risk factor for post-PCNL sepsis (27). There is no consensus about the threshold stone size that implies a higher risk of infectious complications. Many studies assessed stone size with a cutoff of 20 or 25mm. However, this dimension is usually associated with the stone size for PCNL indication, making it difficult to distinguish between risk groups. Therefore, our finding of a cutoff of 35 mm as a risk factor for urinary septic shock helps to identify high infectious risk patients more effectively.

Bacteriuria diagnosed in a preoperative urine culture is a risk factor for urinary septic shock. Moreover, the isolation of E. coli and gram-negative bacilli (GNB) also demonstrated a statistically significant risk association. Nowadays, there is evidence that urine culture collected directly from the renal pelvis is considered more indicative of the causative organism of sepsis (28). Coagulase-negative Staphylococcus was the most frequently isolated agent from the culture of the stones. However, it was not associated with any cases of urinary septic shock, in contrast to the isolation of E. coli, which was associated with two patients experiencing this outcome. These findings may indicate contamination rather than the development of an infection.

Previous studies also demonstrated that preoperative infected urine is a risk factor for infection after PCNL. Xun *et al*. conducted a retrospective study with

745 patients, in which positive urine culture (OR 3.24, p=0.025) was associated with sepsis (29). A meta-analysis published by Lai et al., based on 12 prospective studies (n=1348), concluded that positive preoperative urine culture (OR 2.14, p=0.026), positive intraoperative renal pelvis culture (OR 8.27, p=0.0001) and positive stone culture (OR 5.68, p=0,0001) were predictive factors for postoperative infection (30). The Clinical Research Office of the Endourological Society (CROES) collected prospective data from 5,803 patients treated with PCNL. The authors demonstrated that postoperative fever (≥ 38.5°C) is associated with positive urine culture (especially GNB), *diabetes mellitus*, the presence of a staghorn calculus, and a preoperative nephrostomy tube (5). In our study, neither hydronephrosis nor previous urinary drainage (ureteral stent or nephrostomy) demonstrated an association with urinary septic shock.

Antibiotic prophylaxis may be associated with a reduction in postoperative infectious outcomes. A recent meta-analysis demonstrated a decrease in the risk of sepsis in the postoperative period of PCNL with the use of oral antibiotic prophylaxis for one week before surgery, regardless of the patient risk factors (15). However, this is still a controversial topic in the literature, and the lack of identification of high-risk patients for postoperative infectious complications limits the optimal use of antibiotics in patients who would benefit the most from treatment.

Our study has some limitations. This is a retrospective study from a single institution. Nevertheless, the study was based on a large, prospectively filled electronic medical records database. PCNL was performed by different surgeons in this retrospective study. Variability in surgical proficiency among different surgeons may impact the results of the study outcomes, as in real life different surgeons may have different outcomes of the same procedure. Additionally, the rarity of the primary endpoint prevents us from prospectively assessing the risk factors for urinary septic shock in patients undergoing PCNL.

CONCLUSION

Patients with stones larger than 35mm, positive preoperative urine culture, and a higher CCI are at higher risk for urinary septic shock after PCNL. These findings are of utmost importance to optimize the perioperative care of these patients to prevent life-threatening complications.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Alexandre Danilovic, MD
Av. Dr. Eneas de Carvalho Aguiar, 255, 7 andar Sala 7175
São Paulo, SP, 05403-000, Brasil
Telephone: +55 11 2661-8080
E-mail: alexandre.danilovic@hc.fm.usp.br

ORIGINAL ARTICLE

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A nomogram model for the occurrence of bladder spasm after TURP in patients with prostate enlargement based on serum prostacyclin and 5-hydroxytryptamine and clinical characteristics

Pengfei Shang [1], Miaomiao Lan [2]

[1] Department of Urology, Heji Hospital Affiliated to Changzhi Medical College, Changzhi, Shanaxi, PR. China; [2] Department of Obstetrics, Heping Hospital Affiliated to Changzhi Medical College, Changzhi, Shanaxi, PR. China

ABSTRACT

Objective: With the development of analytical methods, mathematical models based on humoral biomarkers have become more widely used in the medical field. This study aims to investigate the risk factors associated with the occurrence of bladder spasm after transurethral resection of the prostate (TURP) in patients with prostate enlargement, and then construct a nomogram model.

Materials and methods: Two hundred and forty-two patients with prostate enlargement who underwent TURP were included. Patients were divided into Spasm group (n=65) and non-spasm group (n=177) according to whether they had bladder spasm after surgery. Serum prostacyclin (PGI2) and 5-hydroxytryptamine (5-HT) levels were measured by enzyme-linked immunoassay. Univariate and multivariate logistic regression were used to analyze the risk factors.

Results: Postoperative serum PGI2 and 5-HT levels were higher in patients in the Spasm group compared with the Non-spasm group (P<0.05). Preoperative anxiety, drainage tube obstruction, and elevated postoperative levels of PGI2 and 5-HT were independent risk factors for bladder spasm after TURP (P<0.05). The C-index of the model was 0.978 (0.959-0.997), with a $\chi 2 = 4.438$ (p = 0.816) for Hosmer-Lemeshow goodness-of-fit test. The ROC curve to assess the discrimination of the nomogram model showed an AUC of 0.978 (0.959-0.997).

Conclusion: Preoperative anxiety, drainage tube obstruction, and elevated postoperative serum PGI2 and 5-HT levels are independent risk factors for bladder spasm after TURP. The nomogram model based on the aforementioned independent risk factors had good discrimination and predictive abilities, which may provide a high guidance value for predicting the occurrence of bladder spasm in clinical practice.

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Miaomiao Lan
https://orcid.org/0009-0001-3141-4914

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INTRODUCTION

Prostate enlargement is a common clinical male urological disorder that is mostly observed in older population (1). The symptoms of prostate enlargement include difficult urination, urgent urination, frequent urination, nocturia, and interrupted urination, which seriously affect the quality of life of patients (2). Transurethral resection of the prostate (TURP) is a common surgical procedure for prostate enlargement with the advantages of less invasiveness and faster postoperative recovery (3). Postoperative bladder spasm is a common complication of TURP, which refers to the spastic contraction of the detrusor of the bladder, manifesting as lower urinary tract symptoms, such as urinary urgency, temporary urinary closure, and lower abdominal holding pain; in severe cases, secondary bleeding, poor drainage, and urinary tract infection (4, 5). More studies have been conducted on the factors that influence bladder spasm after TURP. However, achieving individualized predictions remains an urgent clinical challenge.

However, the pathogenesis of bladder spasms remains unclear. Studies on clinical symptoms and urodynamic findings have confirmed that detrusor instability is the most important cause of urinary frequency, urgency, and urge incontinence. Bladder detrusor instability and bladder overactivity may be influenced by neurotransmitter release or inflammatory mediators (6-8). Prostacyclin (PGI2) is an important vasoactive substance produced in bladder tissue and plays a key role in bladder homeostasis and inflammation (9). 5-Hydroxytryptamine (5-HT) is as important regulatory neurotransmitter that has been shown to be involved with the regulation of normal bladder voiding function and detrusor overactivity (10, 11).

The application of mathematical models based on humoral biomarkers in medicine has increased with the advancement of analytical techniques. Less research has been done on the use of serum markers to forecast the likelihood of bladder spasms following TURP. In this study, we investigated the factors associated with the occurrence of bladder spasm after TURP surgery by analyzing serum PGI2 and 5-HT levels, and other clinical indicators. In addition, this study established a nomogram model based on the risk factors affecting postoperative bladder spasm after TURP, and serum PGI2 and 5-HT levels to provide reference values to reduce the occurrence of postoperative bladder spasms. We hope to provide new perspectives for recognizing the incidence of postoperative bladder spasms.

MATERIALS AND METHODS

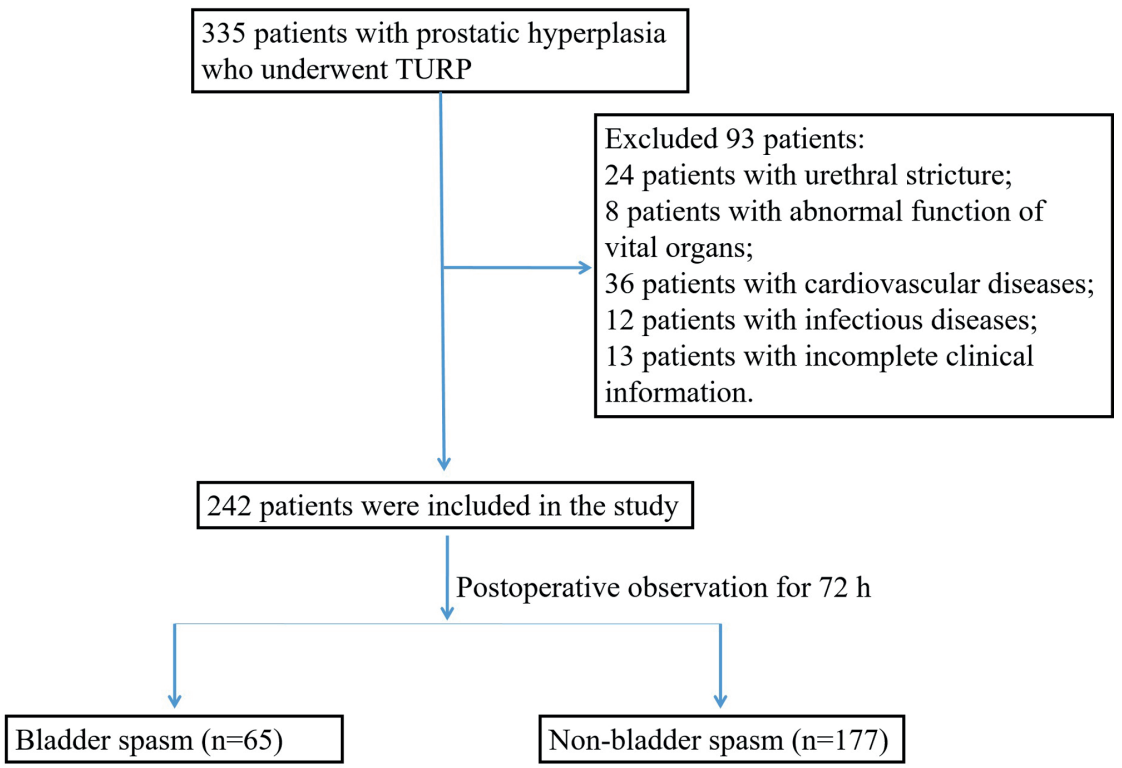
General Information

Clinical data of 242 patients with benign prostate enlargement who underwent TURP from May 2020 to August 2022 were retrospectively analyzed (Figure-1). According to the Chinese Diagnostic and Treatment Guidelines for Urological Diseases (2014), bladder spasm was diagnosed when paroxysmal pubic pain, frequent urination, and painful urination occurred within 72 h after TURP. Sixty-five patients who developed bladder spasm were in the spasm group, aged 40 to 72 years, with a mean of (53.92±12.10) years. The other 177 patients without bladder spasm were in the non-spasm group, aged 21-76 years, with a mean of (55.11±10.82) years. The inclusion criteria were prostate enlargement meeting the diagnostic criteria of the Chinese Diagnostic and Treatment Guidelines for Urological Diseases (2014), International Prostate Symptom Score (IPSS) ≥7, degree of enlargement II to III, compliance with the indications for TURP surgery, absence of abnormal postoperative vital signs, age ≥ 18 years, and complete clinical data. Exclusion criteria included contraindications to surgery, preoperative application of drugs affecting bladder function, history of pelvic surgery, history of combined bladder disease or urethral stricture, concomitant abnormal function of vital organs, cardiovascular disease, infectious disease, comorbid psychiatric disorders, or cognitive dysfunction. The study met medical ethics standards and was approved by the ethics committee of the institution (2020-CZ-006).

Surgery

All surgeries were performed by the same surgeon. TURP was performed in all patients. All procedures were performed under spinal anesthesia by

Figure 1 - The inclusion criteria flowchart of recruited patients.



an experienced surgeon using a 24-Fr resectoscope (Richard Wolf, Germany), a bipolar generator, and saline irrigation fluid. The procedure was performed according to standard methods. Postoperatively, balloon catheters F18–F20 were left in place, and bladder irrigation was performed for 1-3 d, and patients received the same epidural self-controlled analgesia. The catheter was removed without bleeding. Bladder spasm was observed 72 h after surgery.

Data collection

Clinical data of the patients were collected, including general information, such as age, body mass index (BMI), education, annual intake, disease duration, proportion of unstable bladder, and perioperative information. Preoperative anxiety was evaluated using the Self-rating Anxiety (SAS) (12). SAS consists of twenty questions which are scored as 1–4 points individually, resulting in a raw score of 20–80 points; subsequently the standard score is calculated by int (1.25 raw score) and ≥ 60 was considered to have anxiety. An involuntary contraction of the urethral muscle is defined as unstable bladder based on the presence of a bladder pressure ≥ 1.47 kPa during bladder filling on urodynamic examination, which cannot be actively inhibited.

Serum indicators

Five milliliters of early morning fasting venous blood were collected from the patients preoperatively and 24 h postoperatively. After centrifugation at 3000 rpm for 10 min, serum was collected. Serum levels of PGI2 (MBS2501804; MyBioSource, California, USA) and 5-HT (SEKSM-0016; Solarbio, Beijing, China) were measured using enzyme-linked immunosorbent assay. All operations were performed according to the manufacturer's instructions. The corresponding standard curves were derived from the standard concentrations and absorbance values. Concentrations of PGI2 and 5-HT in the samples were determined using a curve equation.

Statistical analysis

The measurement data are described as mean±SD, and two independent sample t-tests were used for comparison between groups. Count data are described as the number of cases, and comparisons between groups were performed using the $\chi 2$ test or Fisher's exact probability method. The predictive value of serum PGI2 and 5-HT levels for the occurrence of postoperative bladder spasm was assessed using a receiver operating characteristic (ROC) curve. Univariate and multivariate logistic regression models were used to screen for the risk factors for the development of postoperative bladder spasms after TURP. A nomogram model for predicting the occurrence of postoperative bladder spasm after TURP was constructed using the rms package in the R software. The bootstrap method was used to repeat sampling 100 times for internal validation. The discrimination ability of the nomogram model was assessed using the area under the ROC curve (AUC). The Hosmer-Lemeshow goodness-of-fit test, calibration curve, and decision curve analysis (DCA) were used to assess the discrimination and accuracy of the model. Differences of P<0.05 was considered statistically significant.

RESULTS

General Information

Bladder spasm occurred in 65 (26.86%) of the 242 patients with prostate enlargement after TURP. Age, BMI, education, yearly intake, length of illness, percentage of unstable bladder, catheter retention time, resected prostate volume, and operational time did not significantly differ between the spasm group and the non-spasticity group (P>0.05). The proportion of patients with preoperative anxiety, rinse fluid temperature <34°C, rinse fluid speed <60 or >80 drops/min, ureteral balloon injection >20 mL, drain blockage, postoperative constipation, and bladder bleeding was significantly higher in the spasm group than in the non-spasm group (P<0.05) as shown in Table-1.

Diagnostic value of serum PGI2 and 5-HT levels on the occurrence of bladder spasm after TURP surgery

There were no significant differences in preoperative serum PGI2 (310.63±85.07 vs. 309.02±91.41, t=0.128, p=0.898) and 5-HT (89.61±19.55 vs. 58.74±18.38, t=0.312, p=0.756) levels between the spasm and non-spasm groups (Figure-2A). Postoperative serum PGI2 (461.34±95.29 vs. 325.89±72.30, t=11.81, p<0.001) and 5-HT (120.09±40.16 vs. 63.06±20.67, t=14.419, p<0.001) levels were markedly higher in patients in spasm group than in the non-spasm group (Figure-2B). ROC analysis showed that the area under the curve (AUC) for PGI2 in predicting the occurrence of bladder spasm after TURP was 0.871 (0.822-0.911), with a specificity of 86.15% and sensitivity of 69.49% (Figure 3A). The AUC of 5-HT for predicting the occurrence of bladder spasm after TURP was 0.900 (0.855-0.935), with a specificity of 78.46% and a sensitivity of 90.40% (Figure-3B). The AUC for predicting the occurrence of bladder spasm after TURP was 0.968 (0.938-0.986) with a specificity of 90.77% and a sensitivity of 82.66% when the two tests were combined (Figure-3C). The diagnostic efficacy of this combination was better than that of PGI2 or 5-HT alone.

Univariate and multivariate logistic regression analysis

Postoperative bladder spasms following TURP were found to be associated with a number of factors, including preoperative anxiety, rinse fluid temperature and speed, ureteral balloon injection, obstruction of the drainage tube, postoperative constipation, bladder bleeding, and elevated postoperative serum PGI2 and 5-HT levels (P<0.05, Table-2). With the occurrence of postoperative bladder spasm as the dependent variable, variables that were significant in the univariate analysis were included in multivariate logistic regression analysis (Table-2). The results showed that preoperative anxiety, drainage tube obstruction, and elevated postoperative serum PGI2 and 5-HT levels were independent risk factors for postoperative bladder spasm after TURP (P<0.05).

Establishment of nomogram model

Based on the outcomes of multivariate logistic regression analysis, a nomogram prediction model was created (Figure-4). The degree of influence of each factor on postoperative bladder spasm is presented as a

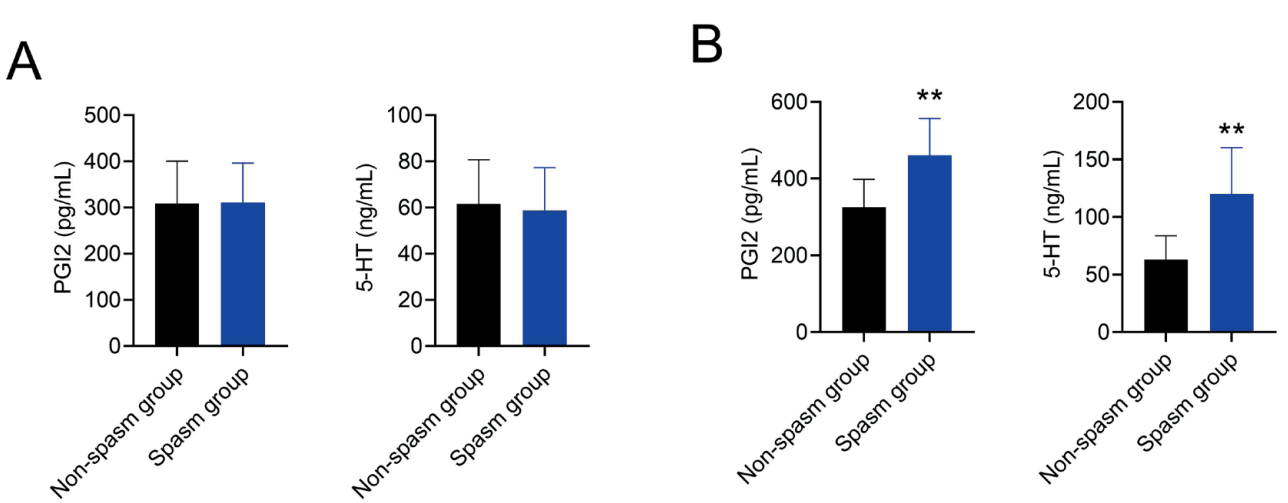
Table 1 - Clinical Characteristics of the Patients at Baseline.

Indicators	n	Non-spastic group (n=177)	Spastic group (n=65)	t/ 2	P
Age (years)	177	55.11±10.82	53.92±12.10	0.734	0.464
BMI (kg/cm^2)	65	23.69±5.39	24.95±4.42	1.694	0.092
Education				1.282	0.286
High School and below	159	120(67.80%)	39(60.00%)		
College and above	83	57(32.20%)	26(40.00%)		
Disease duration				0.056	0.872
≥3 years	174	128(72.32%)	46(70.77%)		
<3 years	68	49(27.68%)	19(29.23%)		
Annual family income				1.083	0.582
<50,000	122	92(51.98%)	30(46.15%)		
50~100,000	77	53(29.94%)	24(36.92%)		
>100,000	43	32(18.08%)	11(16.92%)		
Pre-operative anxiety				10.641	0.002
No	172	136(76.84%)	36(55.38%)		
Yes	70	41(23.16%)	29(44.62%)		
Unstable bladder				2.68	0.102
No	192	145(81.92%)	47(72.31%)		
Yes	50	32(18.08%)	18(27.69%)		
Resected prostate volume				0.194	0.752
<80 mL	169	125(70.62%)	44(67.69%)		
≥80 mL	73	52(29.38%)	21(32.31%)		
Operation time				0.109	0.734
<1 h	186	137(77.4%)	49(75.38%)		
≥1 h	56	40(22.6%)	16(24.62%)		
Catheter retention time				0.677	0.713
1 d	44	34(19.21%)	10(15.38%)		
2 d	55	41(23.16%)	14(21.54%)		
3 d	143	102(57.63%)	41(63.08%)		
Rinse fluid temperature				11.942	0.001
≥20°C	161	129(72.88%)	32(49.23%)		
<20°C	81	48(27.12%)	33(50.77%)		

Rinse liquid speed				7.376	0.009
60~80 drops/min	163	128(72.32%)	35(53.85%)		
80 drops/min.	79	49(27.68%)	30(46.15%)		
Urinary catheter airbag injection water				10.941	0.002
≤20 mL	191	149(84.18%)	42(64.62%)		
>20 mL	51	28(15.82%)	23(35.38%)		
Blockage of drainage tube				8.926	0.004
No	206	158(89.27%)	48(73.85%)		
Yes	36	19(10.73%)	17(26.15%)		
Post-operative constipation				11.119	0.001
No	163	130(73.45%)	33(50.77%)		
Yes	79	47(26.55%)	32(49.23%)		
Bladder bleeding				5.683	0.021
No	161	110(62.15%)	51(78.46%)		
Yes	81	67(37.85%)	14(21.54%)		

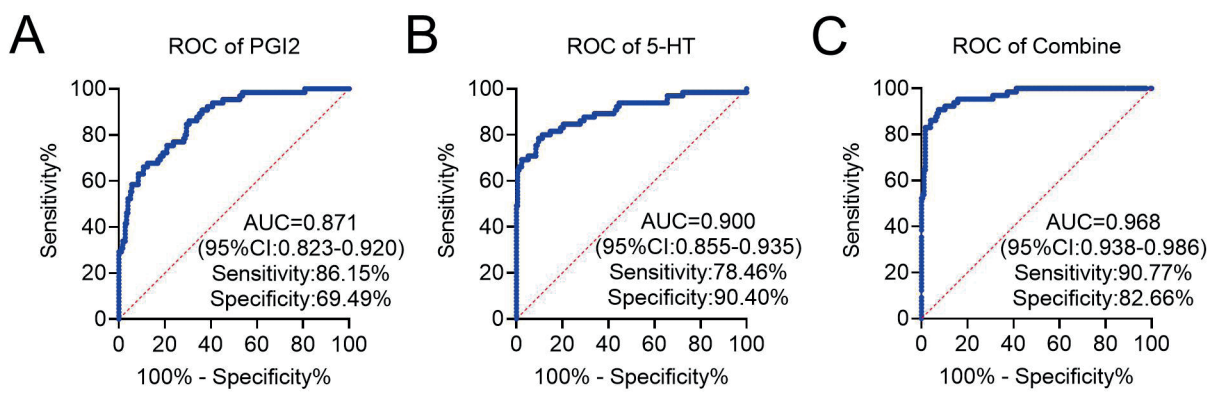
BMI = body mass index.

Figure 2 - Comparison of serum PGI2 and 5-HT levels in the spasm and non-spasm groups.



(A) Preoperative. (B) Postoperative. *P<0.05, compared with non-spasm group.

Figure 3 - The receiver operating characteristics (ROC) curve and area under the ROC curve (AUC) for predicting the occurrence of postoperative bladder spasm. (A) PGI2. (B) 5-HT. (C) PGI2 combined with 5-HT.



score. The nomogram showed scores of 90.75 for PGI2 level, 100 for 5-HT level, 10 for preoperative anxiety, and 12.5 for drainage tube obstruction, respectively.

Validation of the nomogram model

The internal validation of the nomogram model was performed using the bootstrap method with 1000 repeated samples. The results showed that the predicted values of the calibration curve of the nomogram model were generally consistent with the actual values (Figure-5A). The C-index of the model was 0.978 (0.959-0.997), suggesting that the model had high diagnostic value. The Hosmer-Lemeshow goodness-of-fit test had $\chi 2 = 4.438$, P= 0.816. The ROC curve used to assess the discrimination ability of the nomogram model showed an AUC of 0.978 (0.959-0.997) (Figure-5B). The clinical diagnostic value of the model was validated using DCA (Figure-5C). The net benefit of using the nomogram to predict the risk of postoperative bladder spasm was greater when the predictive value of the nomogram model ranged from 0.1–1.0, indicating that the model had a high assessment value.

DISCUSSION

Patients with prostate enlargement frequently experience bladder spasms after surgery. These conditions can impair wound healing, induce paroxysmal suprapubic discomfort, and potentially result in secondary bleeding and urinary tract infections, all of which can hinder recovery (13). Previous studies have shown that the incidence of bladder spasm after TURP ranges from 15.79% to 55.71% (14). The present study showed that the incidence of post-TURP bladder spasm in patients with prostate enlargement was 26.86%, consistent with previous reports. Important to cite that bladder spasms, or postoperative pelvic pain, cause is still unclear, and the symptoms may last longer in about 15% of the cases, influencing the patients' quality of life (15). It is clinically important to identify risk factors associated with the occurrence of postoperative bladder spasms.

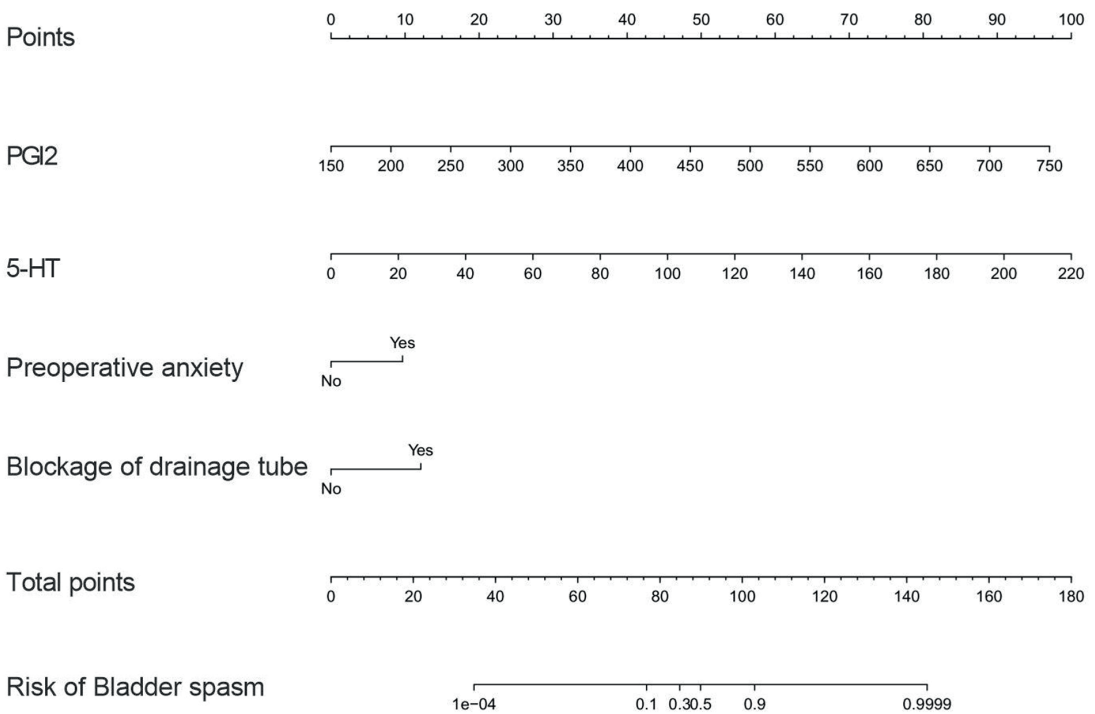
Several causative factors can lead to bladder spasms. In this study, we found that preoperative anxiety, rinse fluid temperature, rinse fluid speed, urethral balloon injection, drainage tube blockage, postoperative constipation, and bladder bleeding were associated with the development of bladder spasms after TURP. Emotions such as anxiety and fear can decrease sympathetic tone, and detrusors are subjected to inhibitory effects from the pituitary-hypothalamic-adrenocortical system, leading to decreased bladder stability (16). Furthermore, poor extraction and bleeding from the indwelling line are likely to result from increased production of bradykinin and other chemicals, which raises the risk of bladder spasm. For postoperative bladder care, the temperature and rinse rate during bladder irrigation are crucial (14, 17). Low rinse fluid temperature not only causes irritation in bladder smooth muscle but also causes hypothermia in patients.

Table 2 - Univariate and multivariate analysis of the risk of bladder spasms.

		Univariate			Multivariate	
Indicators	OR	95%CI	P	OR	95%CI	P
Age	0.990	0.965-1.016	0.464			
BMI	1.050	0.992-1.112	0.093			
Education	1.404	0.780-2.527	0.258			
Disease duration ≥ 3 years	1.079	0.576-2.021	0.812			
Annual family income						
50,000~100,000	1.389	0.736-2.619	0.310			
>100,000	1.054	0.474-2.345	0.897			
Preoperative anxiety	2.672	1.465-4.873	0.001	5.026	1.294-19.521	0.020
Unstable bladder	1.735	0.893-3.373	0.104			
Resected prostate volume	1.147	0.622-2.116	0.660			
Catheter retention time						
1d	1.00					
2d	1.034	0.622~2.243	0.686			
3d	1.061	0.637~2.476	0.661			
Operation time ≥ 1 h	1.118	0.575-2.175	0.742			
Rinse fluid temperature < 20℃	2.771	1.539-4.992	0.001			
Rinse liquid speed < 60 or >80 drops/min	2.239	1.243-4.033	0.007			
Urinary catheter air bag injection water > 20 mL	2.945	1.420-6.110	0.004			
Blockage of drainage tube	2.914	1.523-5.578	0.001	7.598	1.919-30.083	0.004
Postoperative constipation	2.682	1.487-4.837	0.001			
Bladder bleeding	0.451	0.232-0.876	0.019			
PGI2	1.020	1.015-1.026	<0.001	1.027	1.017-1.038	<0.001
5-HT	1.071	1.051-1.091	<0.001	1.079	1.050-1.109	<0.001

Moreover, a low rinse fluid temperature may lead to decreased prothrombin activity and aggregation of coagulation factors in the body, thereby increasing the risk of catheter blockage. Therefore, intraoperative bladder rinse fluid should be preheated to approximately 30°C before use and the patient's bladder status should be monitored during the rinse procedure. In this study, bladder irrigation fluid during bladder irrigation was injected at a rate of 60–80 drops/min. A slower speed leads to a poor flushing effect and may cause poor drainage and increased pressure in the bladder. In contrast, a fast bladder irrigation speed can affect smooth muscle contraction and stimulate detrusor while elevating bladder sympathetic excitability (18). Excessive filling of the urethral balloon increases the pressure in the bladder neck and urethra, which in turn stimulates the bladder sensory nerves and causes bladder contraction. Wilde et al. (19)

Figure 4 - Nomogram based on clinical factors for predicting bladder spasm after TURP in patients with prostatic hyperplasia.

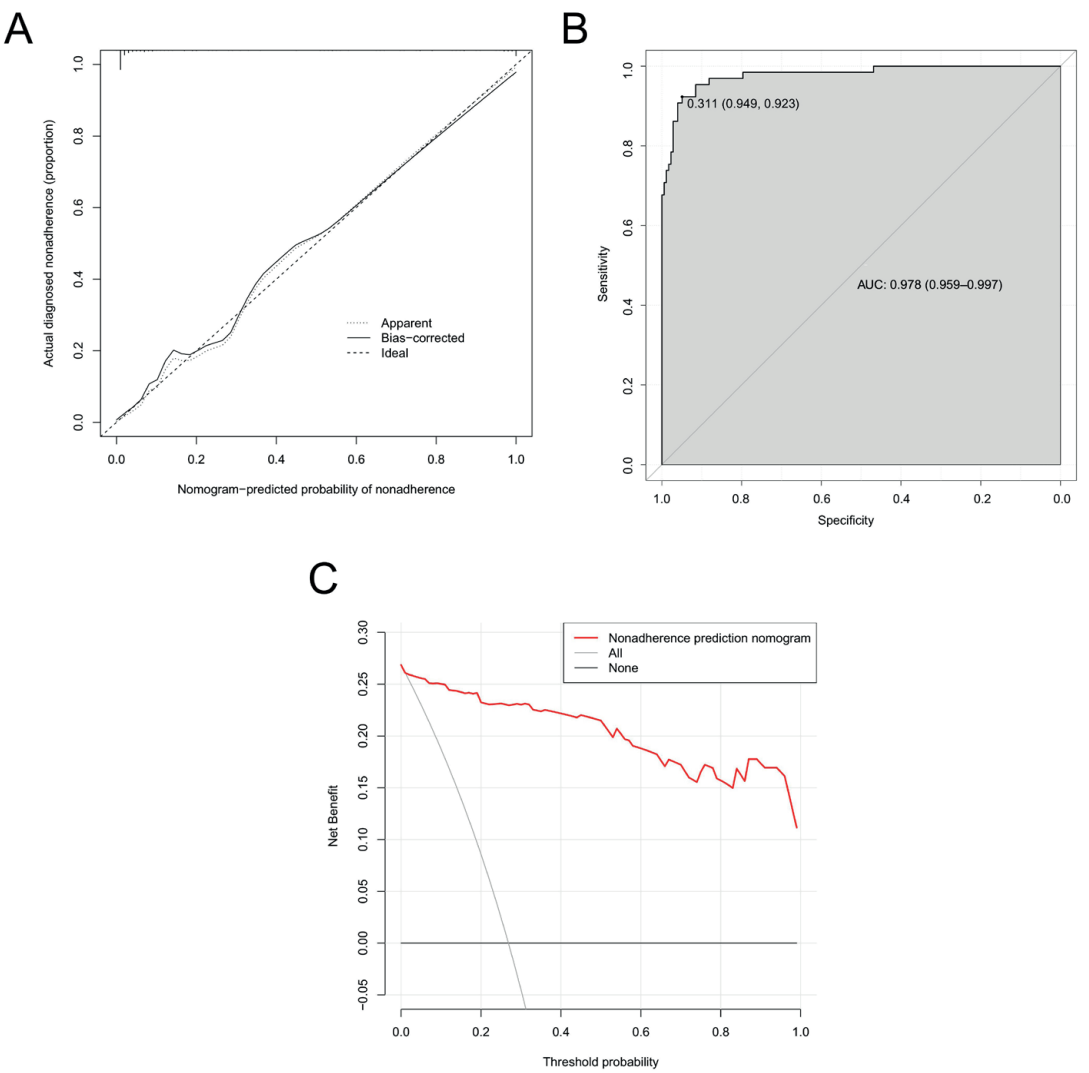


showed that drainage tube blockage can cause catheter-associated urinary tract infections, and urinary tract which can produce inflammatory stimulation of the bladder triangle, in turn inducing bladder spasm. Therefore, it is clinically necessary to ensure that the drainage tube is patent to avoid tortuosity, folding, and blockage. In addition, alterations in the ultrastructure of the hypocompliant or unstable bladder result in electro-coupled depolarization between detrusor cells. As a result, detrusors are prone to persistent contractions during urination, which in turn induces bladder spasm (20, 21). However, this study did not find a difference in the proportion of patients with unstable bladders between the spasm and non-spasm groups, which may be related to the small sample size.

PGI2 is produced by cyclooxygenase-2 (COX-2) and plays an important role in the regulation of the lower urinary tract function. Dilation during detrusor filling, bladder inflammation, mucosal injury, and muscarinic receptor stimulation can induce PGI2 expression in bladder tissue (22). By stimulating capsaicin-sensitive afferent neurons to reduce the stimulus threshold necessary to cause bladder contractions, overexpression of PGI2 contributes to the voiding reflex. Urinary symptoms associated with bladder outlet blockage and/or intravesical alterations can cause the bladder's PGI2 to rise. This elevated PGI2 then contributes to the development of symptoms related to the lower urinary tract, including urgency, frequency, and incontinence. Therefore, patients with irritative bladder symptoms such as urinary frequency and urgency can be assessed for lower urinary tract function through alterations in PGI2 release (23).

Neurotransmitter and vasoactive compound 5-HT is extensively diffused in the central nervous system and peripheral tissues. It has been demonstrated that the voiding regulation center, which is housed in the hypothalamus, regulates the lower urinary tract by combining the actions of many neurotransmitters, including 5-HT (24). Andersson et al. (25) demonstrated that 5-HT levels in the brain are directly related to lower urinary tract function. Numerous 5-HT-containing downstream neurons innervated to the

Figure 5 - Evaluation of the nomogram model.



(A) The calibration curves for evaluating the accuracy of the nomogram. (B) The receiver operating characteristics (ROC) curve and area under the ROC curve (AUC) for nomogram. (C) The determination of decision points via Decision Curve Analysis (DCA).

spinal nucleus are received by the autonomic nucleus of the lumbosacral segment along with the somatic nucleus. Multiple 5-HT receptors are dispersed at locations that process afferent and efferent impulses to the lower urinary tract. 5-HT can either cause or prevent voiding, depending on these receptors (26). Overactive bladder illness has been treated with 5-HT reuptake inhibitors as an adjuvant (10). Alterations in 5-HT levels have been associated with the development of depression and anxiety (27), and psychiatric disorders such as depression and anxiety both trigger the development of postoperative bladder spasms (28). In this study, we found that the postoperative serum PGI2 and 5-HT levels were significantly higher in patients with bladder spasms than in those without spasms. In addition, the predictive value of both was higher for the occurrence of postoperative bladder spasms, and their diagnostic efficacy was higher when combined than when used alone. Therefore, serum PGI2 and 5-HT levels can be used as non-invasive diagnostic markers for diagnosing postoperative bladder spasm after TURP.

The nomogram approach allows healthcare practitioners to more easily forecast bad events on an individual

basis by presenting the risk variables filtered by logistic regression analysis as scores. This allows for a visual assessment of each risk factor's contribution to adverse events (29, 30). Plotting nomograms using publicly accessible clinical data and blood biochemical markers is a commonly utilized method for risk assessment of adverse event occurrence (31, 32). For instance, a nomogram model that predicts a poor prognosis for patients receiving radical cystectomy for uroepithelial carcinoma may be created by combining systemic inflammatory response indicators with clinicopathological data (33). Nevertheless, there are currently no published prediction algorithms that accurately forecast the likelihood of bladder spasms following TURP. In this study, we found that preoperative anxiety, drainage tube occlusion, and elevated postoperative serum PGI2 and 5-HT levels are independent risk factors for the development of postoperative bladder spasm after TURP. Importantly, the nomogram model developed based on serum PGI2 and 5-HT levels and the independent risk factors affecting postoperative bladder spasm after TURP showed good precision and discrimination. When the established prediction model was validated, it was found to have good efficacy in terms of the prediction accuracy, calibration curve, and DCA. Therefore, the nomogram model constructed in this study is a valuable tool for assessing postoperative bladder spasm. Additionally, bladder spasm is a difficult to treat condition. Maybe identifying individuals prone to develop this condition may trigger early treatment. Nevertheless, treatment still needs to be further evaluated and the urological Community treats this situation in different manners (15).

To the best of our knowledge, this study was the first to construct a mathematical model for predicting the occurrence of bladder spasms based on humoral biomarkers versus clinical parameters and showed additional clinical benefit. However, this study has several limitations. First, this was a single-center study with a small sample size and a limited population size, reflecting clinical characteristics. Second, as a retrospective analysis, the study population was not randomly included, and the case screening process was subjectively biased, which may have influenced the results. Third, the nomogram model was only used for internal validation, and it could not be determined whether the extrapolation of the model was good. Moreover, as a retrospective analysis, this study used very strict inclusion and exclusion criteria, which to some extent ensured a high degree of consistency in the characteristics of the population and the accuracy of the model, but this advantage also limited the scope of use of the model. The involuntary contraction of the bladder detrusor muscle is regulated by acetylcholine M-type receptors. Study has shown that before awakening from anesthesia after surgery, 2% lidocaine 10 mL and atropine 0.5 mg are injected through the catheter. Lidocaine reduces the sensitivity of the bladder mucosa through local anesthesia, and atropine can specifically block the transmission of acetylcholine in the nerve endings, and the combination of these two drugs can safely and effectively reduce the incidence of bladder spasm after awakening from anesthesia (34). In terms of postoperative medication, although anticholinergic drugs can effectively relieve the symptoms of bladder spasm, but the systemic adverse effects are obvious, while non-anticholinergic antispasmodic drugs such as resorcinol can prevent postoperative bladder spasm and at the same time reduce the anticholinergic-like adverse effects, such as dry mouth and dry eyes (35). The samples included in this study were all operated on by the same surgeon who performed the relevant procedures with the same anesthesia regimen. In addition, in the postoperative period, the patients all received the same epidural self-controlled analgesia. Therefore, these factors in the perioperative period may not influence our conclusions. Of course, the model constructed in this study does require subsequent prospective studies, which is the direction of our future research.

In conclusion, preoperative anxiety, drainage tube occlusion, and elevated postoperative serum PGI2 and 5-HT levels were independent risk factors for the development of bladder spasm in patients with prostate enlargement. The nomogram prediction model based on the risk factors affecting bladder spasm after TURP was helpful for the risk assessment of bladder spasm occurrence and had a guiding value in avoiding or reducing post-TURP complications.

COMPLIANCE WITH ETHICAL STANDARDS

This study was approved by The Ethics Committee of Heji Hospital Affiliated to Changzhi Medical College (2020-CZ-006). Informed consent was

obtained from the participants for participation in the study, and all methods were performed in accordance with relevant guidelines and regulations.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Miaomiao Lan, MD
Department of Obstetrics,
Heping Hospital Affiliated to Changzhi Medical College
No.271, Taihangdongjie Road, Luzhou District,
Changzhi 046000, Shanaxi, PR. China.
E-mail: lanmiaomiaobd@21cn.com

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Modified penile reconstruction in classic bladder exstrophy: Can complete corporal covering of the urethral closure be achieved using incomplete disassembly technique?

Vasily V. Nikolaev [1, 2], **Nikita V. Demin** [2]

[1] *Pirogov Russian National Research Medical University, Moscow, Russia;* [2] *Clinical and Research Institute of Emergency Pediatric Surgery and Trauma, Moscow, Russia*

ABSTRACT

Purpose: To answer the question of whether it is possible to achieve complete corporal covering of the urethral closure using incomplete penile disassembly in classic bladder exstrophy. We hypothesize that mobilization of the corpora under Buck's fascia, their dorsal translocation through the incisions in Buck's fascia and suturing corporal convex sides above the urethra would allow extend corporal covering of the urethra, reducing the risk of urethra-cutaneous fistula formation.

Materials and Methods: A prospective follow-up on all boys who underwent the modified Cantwell-Ransley primary penile reconstruction was conducted. Inclusion criteria comprised bladder exstrophy closure in our institution, ensuring a postoperative follow-up period of no less than 24 months. The key innovation of the technique lies in a deep dissection of the dependent corpora under Buck's fascia, followed by their dorsal relocation through extended dorsal incisions in Buck's fascia, and limited external corporal rotation 90 degrees only at the base of the penis.

Results: Between November 2019 and March 2022, 18 boys aged 11 to 35 months met the inclusion criteria and underwent the modified penile reconstruction. Surgical procedures and postoperative period did not include any major complications. Total corporal covering of the urethral sutures was achieved in 15 of 18 patients. No urethra-cutaneous fistulas were observed within 2 years of follow-up. All individuals demonstrated spontaneous erections, and the absence of dorsal curvature was documented.

Conclusion: The modified technique of incomplete penile disassembly applied in a homogenous group of patients with classic bladder exstrophy allows penile shaft elongation, improved aesthetic outcomes, preserved erections, and eliminates dorsal curvature. The technique demonstrated feasibility and reliability while maintaining positive effects on tissue circulation. The absence of urethra-cutaneous fistulae is attributed to the complete corporal covering of the urethral sutures and supports the initial hypothesis.

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Vasily Nikolaev
https://orcid.org/0000-0001-7815-4825

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INTRODUCTION

Penile reconstruction in individuals with classic bladder exstrophy (CBE) represents a formidable challenge. The implementation of penile disassembly techniques, involving the ventral relocation of the urethra during the latter part of the 20th century has notably enhanced aesthetic results and diminished complication rates. Despite these advancements, issues such as diminished penile shaft size and recurring dorsal curvature (1, 2) persist as prevalent concerns following epispadias repair, prompting the need for ongoing technical refinements (3).

Currently, Cantwell-Ransley repair modifications or incomplete penile disassembly (IPD) techniques are extensively employed (4-7). In IPD techniques, the cavernous bodies (CB) remain dependent — affixed to the glans penis on one side and to the ischio-pubic rami on the other. The urethral plate is not entirely disassembled from the glans. A distinctive characteristic of these approaches involves the internal rotation of the corpora, generally without the mobilization of neurovascular bundles (NVB). The corpora are approximated at the midline above the urethra by their short concave sides, housing inextensible intrinsic chords. This configuration results straightening and also shortening of the penile shaft (PS) and a deficiency in corporal covering of proximal urethral sutures, a factor believed to be linked to the development of urethracutaneous fistula (8-10). The Manchester group (11) recently reported external rotation of the corpora without Buck's fascia elevating in incomplete penile disassembly, which also did not lead to penile shaft lengthening and complete coverage of the urethral sutures due to lateral tension of the corpora.

We hypothesize that by mobilizing the corpora from Buck's fascia, relocating them over the urethra through the extended dorsal incisions in Buck's fascia, and suturing together the longer ventral corporal surfaces, it would be possible to lengthen and straighten penile shaft and extend the corporal covering of the urethra, reducing the risk of urethracutaneous fistula formation. To implement this idea, several modifications were introduced into the incomplete penile disassembly technique and are presented in this report along with mid-term results.

MATERIALS AND METHODS

The study protocol was approved by the Research Ethics Committee (approval number REB# 1000054434) 19 September 2019.

We conducted a prospective follow-up on all boys with classic bladder exstrophy who underwent primary penile reconstruction by employing a modified technique, ensuring a postoperative follow-up period > 24 months. Inclusion criteria comprised patients with CBE who underwent closure in our institution. Patients presented with very short urethral plates - less than 15 mm between the verumontanum and the tip of the glans were excluded. The detailed technique for exstrophy closure has been previously elucidated (12).

As part of the urinary bladder closure, the Johnston maneuver was executed, involving the separation of the anterior ⅔ of the CB from the ischiopubic rami, along with the periosteum, in preparation for subsequent epispadias repair (ER) (13). Additionally, pubic bones approximation preventing deepening of the penis was performed in conjunction with the described exstrophy closure procedure.

The pre-surgical protocol included the application of testosterone 2–3 times daily for a duration of 3 weeks. For cases of total urinary incontinence, two testosterone enanthate injections (2 mg/kg) were administered five and two weeks prior to the surgery.

Surgical technique

The dissection of the skin was carried out along the borders of the urethral plate, encircling the neo-meatus, and extending along the corona. Lateral incisions of the skin, reaching down to the scrotum, were made to alleviate tissue tension and facilitate unimpeded access to the attachments of CB. We consider the reconstruction of the penile shaft as a simultaneous achievement of several objectives:

maximizing the elongation of the penile shaft and achieving a cylindrical shape; transposing the corpora over the urethra; correcting any dorsal curvature; ensuring coverage of the urethral sutures with the corpora; preserving penile circulation.

Corporal dissection

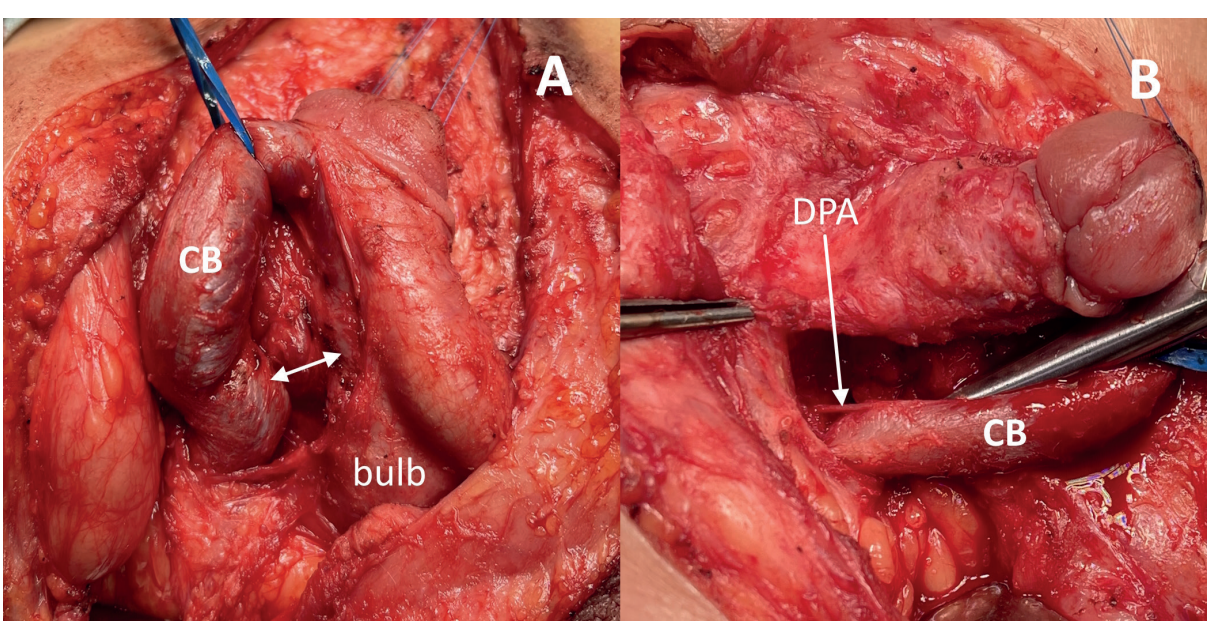
Corporal mobilization was conducted beneath BF through its longitudinal ventral incisions, extending from the glans corona down to the ischiocavernosus muscle (14). To safeguard the neurovascular bundles and the spongious body, they remained attached to BF, ensuring their protection and facilitating the overall technique. The cavernous bodies were deliberately left dependent, retaining their connection to the tip of the glans penis and the ischiopubic rami. In this way, distally the corpora are almost completely released, leaving only the apexes of the corpora connected to the glans (4, 5).

Similarly, the urethral plate was not completely separated from the glans. An important step involved dissecting the corpora from the posterior part of the bulb, where a firm attachment restricted the mobility of CB. This medial corporal attachment was sharply dissected without significant bleeding (Figure-1A). Typically, at this level, the deep artery of the penis dorsally or dorsal-laterally enters the CB (Figure-1B), necessitating preservation due to its vital role in supplying the corpora (15).

Cavernous body relocation and urethroplasty.

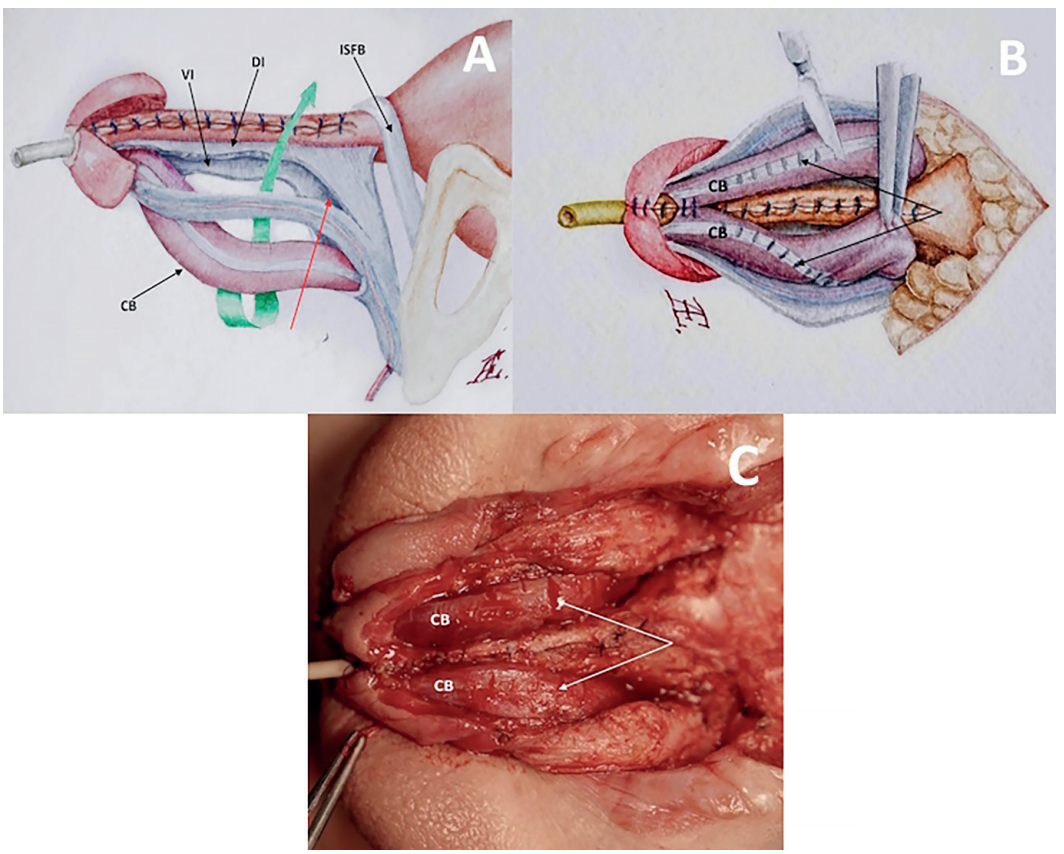
Dorsal incisions were meticulously crafted between the edge of the urethral plate and the NCB, extending from the tip of the glans to the prostatic part of the urethra, with a dorsal cut of BF to the perineum (Figure-2A). It is crucial to note that the BF incision continues more proximally than its junction with the urethral plate, a significant factor in achieving maximal penile shaft length and complete corporal covering of the urethral closure. Tubularization of the urethral plate was executed using a continuous extramucosal 6/0 suture over a #8 Ch pigtail catheter. When the penis is

Figure 1 - Cavernous body dissection.



A) Cavernous body is dissected from the urethral bulb (bulb). White arrow indicates the gap between the bulb and the right CB. B) Deep penile artery (DPA) dorsally enters the cavernous body – white arrow.

Figure 2 - Cavernous body relocation.



A) Dependent CB is prepared to be relocated dorsally along the green arrow. Dorsal incision of Buck's fascia between the neurovascular bundle and the urethral plate stretches from the glans to the pelvic floor level (red arrow) extending more proximally to the junction with the urethral plate. The inter-symphisal fibrous band (ISFB) is located at the level of the pelvic floor. It is apparent that the neurovascular bundle remains attached to Buck's fascia. The urethra is tubularized. DI - dorsal incision, VI - ventral incision. B) Counter torsion of the corpora. After the attenuations on the chordee are made, the convex ventral sides of the corpora are turned medially (as shown with the forceps) at the base of the penis, while no torsion is noted at the apical portion of corpora. The corpora are sutured in the middle of the glans. C) Cavernous bodies were moved dorsally through the incisions in Buck's fascia. Noteworthy is the absence of mid-corporal torsion. Releasing transverse incisions (attenuations) are made on the chordee (arrows).

deflected caudally, CB forms mobile loops, freely relocating dorsally through the extended incisions of BF. To relieve intrinsic chordee, transverse incisions were made across it (16). Subsequently, the convex sides of the corpora at the base of the penis were directed toward each other over the urethra (Figures 2B and C). In the described technique, rotation of the corpora at the base of the penis did not lead to significant torsion in the middle of the shaft or crura of the penis and deterioration of blood supply through the cavernous artery, due to extended proximal and distal corporal mobilization and a relatively small angle of rotation.

Penile shaft assembly

To effectively address the curvature of PS and establish a cylindrical penile shape, a series of Gittes tests are conducted during the assembly process. The initial suture is strategically placed between the convex sides of the corpora over the urethra, positioned more proximally than the initiation point of urethral closure. This suture serves as the proximal edge of the penile shaft.

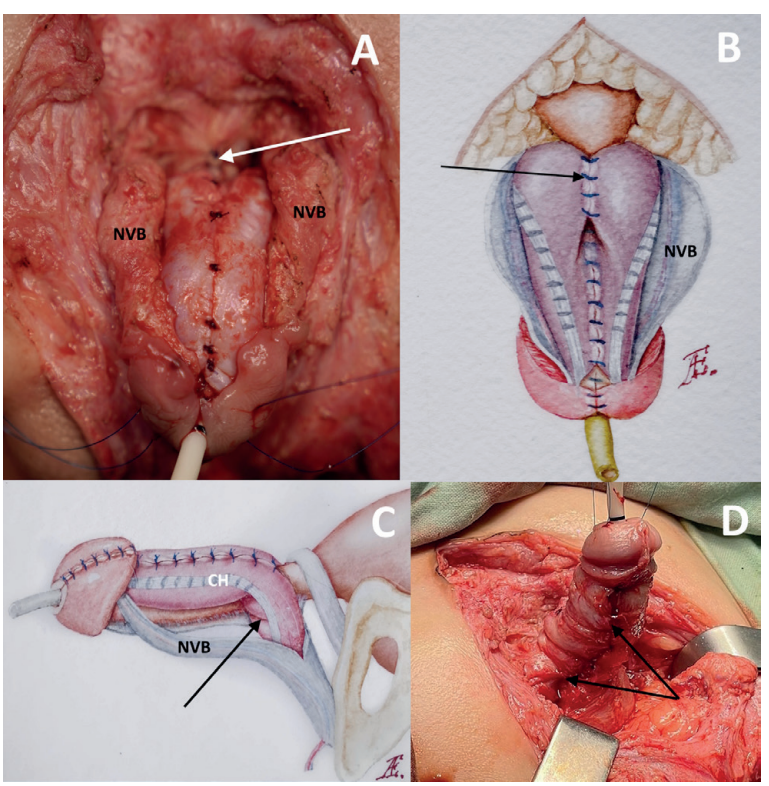
The second suture is delicately placed over the urethra between the corpora in the middle of the glans, with only slight internal corporal rotation in this place ensuring a tension-free application. Notably, the apical

segments of the corpora are deliberately left unsutured to avoid potential circulatory complications in the glans and the distal section of the urethra. The closure of the corpora along the line connecting these two sutures results in the formation of the PS. Therefore, only corporal segments at the base of the penile shaft were rotated externally 90 degrees ensuring covering the urethral sutures below the prostate (Figure-3A). Dorsal intercorporeal sutures are duplicated to reinforce the base of the penis. Subsequently, a conventional glanduloplasty is performed (Figures 3B and C). The restoration of the fascial sheath of the PS is executed without tension to prevent tethering (Figure-3D). Specifically, only the superficial fascia is closed along the midline dorsally, with no approximation of NVB in the midline. For skin coverage, parascrotal skin flaps and penile skin are employed (Figure-4A).

Postoperative assessment

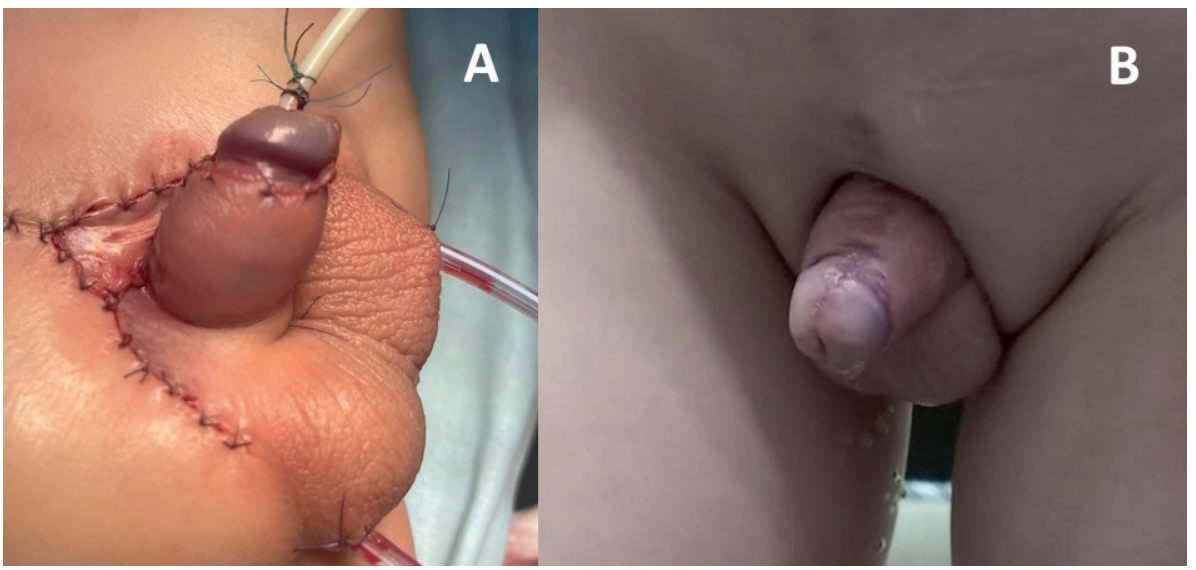
All patients underwent follow-up assessments for 24 months post-surgery (Figure- 4B). The evaluation encompassed the visual appraisal of the penis, its configuration during spontaneous erections, and urination, conducted both in an outpatient setting and through the analysis of photographs and video recordings of urination. Comprehensive examinations of the children occurred at the outpatient facility at intervals of 1, 3, 6, 12, 24 months following surgical intervention.

Figure 3 - Penile shaft assembly.



A and B) The CB are joined along the midline. The base of the penis is reinforced by the security stitches to prevent dehiscence during erections. White arrow - narrowing suture is placed on the prostatic urethra. Black arrow - second row of stitches at the base of the penis. All the sutures of the urethral closure are covered with the CB. C) Side view after penile shaft reconstruction. As a result of relocation and the approximation of the convex sides of the corpora, the normal Γ-shape of the CB is restored. The chordae (Ch) at the base of the penis are located laterally - the curvature is corrected. A cylindrical shape of the penis has been achieved. Black arrow - subtle torsion of the penile crus. D) Sutures attaching Buck's fascia to the albuginea are marked with black arrows. Restored fascial sheath of the penile shaft.

Figure 4 - Results of penile reconstruction at the end of the procedure (A) and at mid-term follow-up (B).



Characteristic features of the appearance of the penis following the implementation of the described technique: visible shaft of the penis, which has a cylindrical shape; lack of curvature; orthotopic position of the meatus; normal angle of deflection of the penis during spontaneous erection.

RESULTS

Between November 2019 and March 2022, penile reconstruction was conducted on 38 patients diagnosed with CBE. Out of this cohort, 18 boys aged 11 to 35 months satisfied the inclusion criteria and underwent the modified primary penile reconstruction and epispadias repair as part of staged repair of bladder exstrophy.

Surgical procedures and postoperative phases were notably devoid of any major complications. The developed surgical technique was performed in a similar manner in this series without osteotomy or pubic bones approximation. Total corporal covering of the urethral sutures was achieved in 15 of 18 patients. In three cases, challenging micturition following catheter removal necessitated the reinsertion of the catheter for an additional two weeks, coupled with a course of antibiotics. Local skin wound dehiscence (Clavien-Dindo - I) without urinary leakage was noted in 5 cases.

Postoperatively, aesthetic outcome was evaluated by two ambulatory urologists as good in 14 and satisfactory in 4 the patients with vertical meatus at the tip of the conical glans, a straight penis, and pliable penile skin (Figure-4). Unobstructed urination was further noted in all boys. Notably, no urethra-cutaneous fistulas were observed within this patient cohort. Twelve boys showed an increase in the volumes of morning micturition, and the duration of dry intervals at rest.

All individuals demonstrated spontaneous erections, and the absence of dorsal curvature was consistently noted and documented through video and photographic evidence during the 24 months post-discharge period (Figures 4A and B). Each patient exhibited a PS that maintained a straight configuration, deviating at an angle of 90±20 degrees from the frontal plane during spontaneous erections. At the midterm follow-up, all cases demonstrated the attainment of a correct cylindrical shape and displayed proper aesthetic features of the penis.

DISCUSSION

Penile reconstruction and ER in patients with CBE significantly advanced in the late 20th century. Such important achievements in reconstructive tech-

niques for epispadias repair include penile disassembly (4, 10) and method for correcting the dorsal curvature of the penis by rotating the corpora, which was invented by Stephen Koff (17). The author used outward rotation of corpora without ventralization of the urethra in Young's epispadias repair. Subsequently, external or internal rotation of the corpora was adopted in most modifications of penile disassembly (4, 5, 18).

Notably, Cantwell-Ransley modifications of incomplete disassembly in epispadias repair, widely employed by experts, yield acceptable results (19). These techniques carry a lower risk of ischemic disorders and avoid hypospadias, distinguishing them from more radical approaches such as the Kelly soft tissue mobilization or the Mitchell Complete Penile Disassembly technique (10, 20-22).

Distinctive features of IPD techniques encompass preserved connection of the glans and the corpora to the urethral plate, coupled with the inward rotation of the corpora over the urethra to eliminate dorsal curvature. While corporal inward rotation successfully corrects curvature in many cases without the mobilization of the NVB, most of modifications (4, 5) involve suturing the short, concave sides of the corpora, which contain the least elastic parts of the albuginea known as intrinsic chordae. This inevitably results in penis shortening. Consequently, the sutures of the tubularized urethra at the base of the penis remain uncovered by the corpora, posing a well-established risk for the dehiscence or urethra-cutaneous fistula formation. (8, 24, 25). Their treatment is difficult and sometimes requires 3 to 6 operations (26). Furthermore, these modifications to the Cantwell-Ransley technique fail to produce a cylindrical penis shape, as the convex sides of the corpora end up on the exterior following inward rotation, potentially causing a waist-like deformity at the base of the penis (23).

Our hypothesis posited that by suturing the longer, convex sides of the dependent corpora over the urethra in incomplete penile disassembly technique such as modified Cantwell-Ransley (4, 5), it would be possible to achieve penile shaft lengthening, covering of urethral sutures, and correct dorsal curvature. This conceptualization was actualized in the described technique, which was uniformly applied in a prospective study involving a homogeneous series of patients undergoing primary ER within 6-11 months after exstrophy closure.

The proposed technique effectively attains the aforementioned goals of penile shaft reconstruction through a series of consecutive steps. The key innovation of this technique lies in a more proximal subfascial corporal dissection for releasing and lengthening of the dependent corpora, followed by their dorsal relocation through extended dorsal incisions in Buck's fascia and limited 90-degree external rotation of the short corporal segments at the base of the penis to avoid a significant mid-corporal torsion and subsequent circulatory problems. In the existing literature, we did not find a description of a technique combining the reconstruction of the penile shaft in a similar manner.

With the CB in a dependent position and the urethra, along with the spongiosum, attached to the glans, achieving free corporal relocation necessitates a deeper mobilization of the corpora from Buck's fascia. A crucial aspect of this dissection involves the complete detachment of the medial surface of the corpora from the posterior aspect of the bulb, entailing the transection of the robust attachment between them. We didn't meet descriptions of this specific anatomical structure or features of the dissection of the corporal-bulbar connection at this particular location. Corporal mobilization to the tip of the glans is also important for free CB relocation and rotation. Achieving penile shaft elongation and the corporal covering above the urethral sutures involves pulling the mobilized CB dorsally through extended dorsal incisions on Buck's fascia. Correction of dorsal curvature and the formation of a cylindrical penile shaft are accomplished by equalizing the length of all corporal sides. This is achieved by orienting the corporal convex sides at the base of the penis toward each other and closing them above the urethra. The chordee which was initially located dorsally, as a result of torsion of the penile crura 90 degrees outward, is moved at the base of the penis to the lateral side, where it ceases to have a bending effect. In this manner, the penis can be fully straightened, the corpora assume their normal Γ-shape, and the bending moment of force curving the corpora dorsally approaches zero. We found that penile curvature correction by corporal rotation is somewhat more complex than described in the literature,

since rotation of any corporal segment causes torsion between the fixed and rotated portions. The shorter the twisted parts and the greater the angle of rotation of the corpora, the greater the deformity and the risk of partial or complete obstruction of the cavernous artery.

In the described technique short rotated segments and extended mobilization of the corpora lengthen twisted areas, reducing local deformation, which apparently has a positive impact on blood supply. Moreover, the corpora mostly cover the urethral suture, extending from the neo-meatus to the center of the glans, thereby minimizing the risk of urethra-cutaneous fistula formation. Our prior experiences have indicated that the approximation of the distal segments of the corpora is not advisable, as it may compromise apical blood supply and lead to glans ischemia. We did not observe any disruption of corporal blood supply after its dissection from the bulb. Obviously, this technical point is delicate, like the technique of dissection of the cavernous bodies in general and requires experience in the penile disassembly techniques.

The penile shaft assembly technique is often overlooked in the literature, despite its pivotal role in determining the final shape and position of the penis. Notably, pronounced tethering can arise when NVB are approximated at the midline, a practice recommended by most authors conducting incomplete or complete penile disassembly. Therefore, our preference is to close dorsally only the superficial fascia while attaching the edges of Buck's fascia to the ventrolateral surface of the albuginea without tension.

The assessment of aesthetic and functional results involved both physical examinations and the review of photographic and video materials captured at rest and during spontaneous erections, provided by the parents. This comprehensive approach aimed to obtain tangible evidence of dorsal curvature correction and the shape of the penis during an erection in individuals with exstrophy. The inclusion of real-life evidence contributed to the verification of the outcomes achieved by the proposed evaluation methodology, making it viable for further evaluation and adoption.

Limitations of this study include a relatively small cohort of patients who underwent this procedure, specifically penile reconstruction as part of staged exstrophy repair, and the absence of a control group. The technique should be used by the surgeons experienced in penile disassembly techniques with caution to avoid circulatory disorders. Obviously, long-term results of the technique after puberty are required, but the mid-term aesthetic and functional results obtained are encouraging.

CONCLUSIONS

The presented modified Cantwell-Ransley technique of incomplete penile disassembly with reconstruction was applied in a homogeneous group of patients with classic bladder exstrophy. The key innovation of the technique lies in the complete corporal detachment from the bulb followed by their dorsal relocation through extended dorsal incisions in Buck's fascia and a limited 90-degree external local corporal rotation at the base of the penis. The technique demonstrated feasibility and reliability while maintaining positive effects on tissue circulation, allowed penile shaft elongation, improved aesthetic outcomes, preserved erections, and eliminated dorsal curvature. The absence of urethra-cutaneous fistulae is attributed to the complete corporal covering of the urethral sutures which supports the initial hypothesis.

ABBREVIATIONS

ER = Epispadias repair
CBE = Classic bladder exstrophy
IPD = Incomplete penile disassembly
CB = Cavernous bodies
NVB = Neurovascular bundles
PS = Penile shaft
BF = Buck's fascia

COMPLIANCE WITH ETHICAL STANDARDS

The study protocol was approved by the institutional Research Ethics Board (approval number REB# 1000054434) 19 september 2019. The research was performed in compliance with the research ethics board requirement.

The patients in this manuscript have given written informed consent to publication of their case details.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Nikita V. Demin, MD
Clinical and Research Institute of
Emergency Pediatric Surgery and Trauma
Bolshaya Polyanka St, 22,
Moscow, Russia, 119180
Telephone: + 7 903 183-0141
E-mail: urodemin@gmail.com

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Validation of the Barcelona-MRI predictive model when PI-RADS v2.1 is used with transperineal prostate biopsies

Juan Morote [1, 2], Nahuel Paesano [2, 3], Natàlia Picola [4], Jesús Muñoz-Rodriguez [5], Xavier Ruiz-Plazas [6], Marta V. Muñoz-Rivero [7], Ana Celma [1], Gemma García-de Manuel [8], Berta Miró [9], Pol Servian [10], José M. Abascal [11, 12]

[1] Department of Urology, Hospital Univeritari Vall d´Hebron, Barcelona, Spain; [2] Department of Surgery, Universitat Autònoma de Barcelona, Bellaterra, Spain; [3] Clínica Creu Blanca, Barcelona, Spain; [4] Department of Urology, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Spain; [5] Department of Urology, Hospital Universitari Parc Tauli, Sabadell, Spain; [6] Department of Urology, Hospital Universitari Joan XXIII, Tarragona, Spain; [7] Department of Urology, Hospital Universitari Arnau de Vilanova, Lleida, Spain; [8] Department of Urology, Hospital Universitari Josep Trueta, Girona, Spain; [9] Statistic Unit, Vall d´Hebron Research Institute, Barcelona, Spain; [10] Department of Urology, Hospital Univeritari Germans Trias i Pujol, Badalona, Spain; [11] Department of Urology, Parc de Salut Mar, Barcelona Spain; [12] Department of Medicine and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain

ABSTRACT

Purpose: To validate the Barcelona magnetic resonance imaging predictive model (BCN-MRI PM) in men with pre-biopsy multiparametric MRI (mpMRI) reported with the Prostate Imaging Reporting and Data System (PI-RADS) v2.1, followed by transrectal and transperineal prostate biopsies.

Materials and Methods: Prospective analysis of 3,264 men with PSA >3.0 ng/mL and/or abnormal digital rectal examination who were referred to ten participant centers in the csPCa early detection program of Catalonia (Spain), between 2021 and 2023. MpMRI was reported with the PI-RADS v2.1, and 2- to 4-core MRI-transrectal ultrasound (TRUS) fusion-targeted biopsy of suspected lesions and/or 12-core systematic biopsy were conducted. 2,295 (70.3%) individuals were referred to six centers for transrectal prostate biopsies, while 969 (39.7%) were referred to four centers for transperineal prostate biopsies. CsPCa was classified whenever the International Society of Urologic Pathology grade group was 2 or higher.

Results: CsPCa was detected in 41% of transrectal prostate biopsies and in 45.9% of transperineal prostate biopsies (p <0.016). Both BCN-MRI PM calibration curves were within the ideal correlation between predicted and observed csPCa. Areas under the curve and 95% confidence intervals were 0.847 (0.830-0.857) and 0.830 (0.823-0.855), respectively (p = 0.346). Specificities corresponding to 95% sensitivity were 37.6 and 36.8%, respectively (p = 0.387). The Net benefit of the BCN-MRI PM was similar with both biopsy methods.

Conclusions: The BCN-MRI PM has been successfully validated when mpMRI was reported with the PI-RADS v2.1 and prostate biopsies were conducted via the transrectal and transperineal route.

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Juan Morote Robles
https://orcid.org/0000-0002-2168-323X

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INTRODUCTION

Risk-stratified prostate cancer (PCa) screening, based on serum prostate-specific antigen (PSA) and magnetic resonance imaging (MRI), is currently recommended by the European Union (1). The new paradigm for PCa screening is focused on the early detection of clinically significant PCa (csPCa) (2). This paradigm change is based on evidence reported by the European Randomized Screening Prostate Cancer study in 2009. In this randomized trial, the hazard ratio for cause-specific death in the screening arm, and its 95% confidence interval, as compared with the control arm, reached a significance of 0.80 (0.65 to 0.98) at 8.8 years of follow up (3). This significant reduction of PCa-specific mortality has been maintained after 22 years of follow up in the Göteborg Randomized Population-Based Prostate Cancer Screening Trial (4). The European Association of Urology currently proposes the use of risk-stratified pathways, based on predictive models, for improving csPCa screening by reducing the demand for MRI exams, maximizing the detection of csPCa, and decreasing unnecessary prostate biopsies and over-detection of insignificant PCa (iPCa) (5, 6).

The Barcelona-MRI predictive model (BCN-MRI PM) for individualizing the risk of csPCa detection in prostate biopsies was developed due to the absence of csPCa risk calculators using the Prostate Imaging Reporting and Data System (PI-RADS) v2.0, and six other independent clinical predictive variables without range limitations, namely: age (years), PCa family history (no vs. yes), type of prostate biopsy (initial vs. repeated), serum PSA (ng/mL), digital rectal examination (DRE: normal vs. suspicious), MRI-derived prostate volume (mL), and PI-RADS score from 1 to 5 (7). The BCN-MRI PM development cohort included 1,486 men, with serum PSA >3.0 ng/mL and/or suspicious DRE, who underwent pre-biopsy multiparametric MRI (mpMRI) reported with PI-RADS v.2.0, followed by 2- to 4-core MRI-transrectal ultrasound (TRUS) fusion-targeted biopsy of PI-RADS >3 lesions and 12-core systematic biopsy, but only a 12-core systematic biopsy in those negative MRI (PI-RADS 1 or 2). This development cohort was prospectively recruited in a single academic institution between 2016 and 2019. Additionally, an external validation was conducted in 946 men, who underwent the same PCa suspicion criteria and diagnostic approach as those in the development cohort, in two centers from the Barcelona metropolitan area within the same period (8). The BCN-MRI risk calculator was designed for the easy and quick assessment of individual risk of csPCa, with the novelty of selecting the appropriate threshold for prostate biopsy decision, free available without cost at the https://mripcaprediction.shinyapps.io/MRIPCaPrediction/ (accessed on March 29, 2024). The BCN-MRI PM has been compared with the prestigious Rotterdam-MRI PM in a head-to-head analysis conducted in the external validation cohort. A better overall performance of the BCN-MRI PM was observed, especially in men with PI-RADS of 3 and 4. Additionally, it was observed that 22% of men included in this analysis presented age, serum PSA, or prostate volume out of the range accepted by the Rotterdam-MRI risk calculator (9).

Current predictive models require validation in populations where they are intended to be applied, even if the event of changes in the characteristics of the population from which the development cohort came or changes in the diagnostic procedure. These validations are necessary to ensure the ongoing accuracy of individual predictions (10).

Two relevant changes have recently been incorporated into the early diagnostic approach to csPCa. First, the PI-RADS v2.1 is currently followed for reporting MRI findings, and second, transperineal route for prostate biopsies is suggested for avoiding the infectious complications of prostate biopsies (11, 12). We hypothesise the BCN-MRI predictive model will be successfully validated in when PI-RADS v2.1 is employed for reporting pre-biopsy MRI, and prostate biopsies conducted even by transrectal and transperineal route. The present study aims to validate the BCN-MRI PM in a csPCa opportunistic screening program where the diagnostic approach employed the PI-RADS v2.1 for reporting MRI, and transperineal or transrectal prostate biopsies.

MATERIAL AND METHODS

Design, setting, and participants

This is a prospective study conducted in 3,264 men with the inclusion criteria of (i) suspicion of PCa based on serum PSA of >3.0 ng/mL and/or suspicious DRE, (ii) pre-biopsy mpMRI reported with PI-RADS v2.1, and (iii) prostate biopsy following the scheme of 2- to 4-core MRI-transrectal ultrasound (TRUS) fusion-targeted biopsies and 12-core systematic biopsy in men with PI-RADS ≥ 3, but only a 12-core systematic biopsy in those with PI-RADS <3. This trial was conducted in ten centers participating in the csPCa early detection program of Catalonia (Spain), a region with 7.9 million inhabitants, between January 1, 2020, and June 30, 2023. Reported cases were consecutive in each participant center. A subset of 2,295 men (70.3%) underwent prostate biopsy in six participant centers where transrectal prostate biopsy was exclusively employed, while 969 (29.7%) underwent biopsies in three other centers exclusively employing transperineal biopsies. The exclusion criteria were men with previous diagnosis of PCa, multifocal high-grade prostatic intraepithelial neoplasia, and atypical small acinar proliferation. Men recruited in one participant center where transperineal prostate biopsies followed a mapping scheme for targeted biopsies were not included in this analysis. This project was approved by the ethics committee of the coordinating center (PRAG02/2020), with participants signing an informed consent.

CsPCa suspicion and diagnostic approach

PCa suspicion was based mostly on a serum PSA >3.0 ng/mL, while 95 men (2.9%) exhibited a suspicious DRE with a serum PSA of 3.0 or lower. Men suspected of having PCa were referred to the nearest participating center of the csPCa early detection program. MpMRI was conducted at each participant center using a 1.5 or 3 Tesla scan with a pelvic phased-array surface coil. The acquisition protocol included T2-weighted imaging (T2W), diffusion-weighted imaging (DWI), and dynamic contrast-enhanced (DCE) imaging, according to the guidelines of the European Society of Urogenital Radiology (13). MpMRI exams were reeded by local expert radiologists reporting with the PI-RADS v2.1 (11). All prostate biopsies were performed using freehand technique and software MRI-TRUS fusion image for targeted biopsies in 42.8%, while cognitive fusion was employed in 67.8%. Uropathologists examined the biopsy material in each pathology department and reported PCa using the International Society of Urologic Pathology grade group (GG) classification. CsPCa was considered when the GG was 2 or higher (14).

Statistical Analysis

Statistical analysis was conducted after harmonization of anonymized datasets. The data were prospectively collected and reported according to the Standards of Reporting for MRI-targeted Biopsy Studies (START) to describe the study population (15). Quantitative variables are described using medians and interquartile ranges (25th–75th percentiles), while qualitative variables are described using numbers and percentages. Quantitative variables were compared between groups using the Mann–Whitney U test. Qualitative variables were compared between groups using Pearson's chi-square test. Relative risk (RR) of csPCa and 95% confidence intervals (CI) were assessed. Calibration of the BCN-MRI PM was conducted for both prostate biopsy routes. Discrimination of csPCa from the BCN-MRI PM in each prostate biopsy group was analyzed with receiver operating characteristic (ROC) curves, and the areas under the curve (AUC) were compared with the DeLong test. Specificities corresponding to selected sensitivities with clinical interest were compared and avoided prostate biopsies and loss of csPCa estimated. Net benefit of the BCN-MRI PM over biopsy all men was evaluated through decision curve analysis (DCAs). A p value of <0.05 was considered statistically significant. The data were analyzed using the Statistical Package for the Social Sciences (version 29.0; IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics of both subsets of men who underwent transrectal or transperineal route for

prostate biopsy are summarized in Table-1. We note that baseline characteristics were similar in both subsets. The median interval from MRI exam to prostate biopsy was 27 days. However, csPCa was detected in 940 men (41%) who underwent transrectal prostate biopsy, and in 445 men (45.9%) who underwent transperineal prostate biopsy (p =0.016). The rates of iPCa detection were 16.3% and 17.4%, respectively (p =0.441).

Calibration of the BCN-MRI PM in both subsets was very good. Similar calibration curves between predicted risks and observed csPCa cases in transrectal and transperineal prostate biopsies were observed. Both calibration curves showed a small over-estimation in lowest predictive probabilities of csPCa and minimal under-estimation in the higher predictive probabilities of csPCa, but both were near the ideal correlation line, Figure-1 A-B.

ROC curves showed AUC (95% confidence intervals) of 0.847 (0.830–0.863) and 0.830 (0.804–0.855), in prostate biopsies conducted through transrectal and transperineal routes, respectively, (p =0.346), Figure-2 A-B. Specificities corresponding to 100%, 97.5%, and 95% sensitivity of the BCN-MRI PM and thresholds were analyzed. The specificity corresponding to 100% sensitivity was 1.3 and 1.8%, from the threshold of 0.36% for transrectal biopsies and 0.49% for transperineal biopsies, p = 0.438. The specificities corresponding to 97.5% sensitivity were 23.7 and 22.6%, from the threshold of 5.11% and 8.58% for transrectal and transperineal prostate biopsies respectively, p = 0395. Regarding the sensitivity of 95%, the specificities of the BCN-MRI-PM corresponded to 37.6 and 36.8%, from the threshold of 9.80 and 16.2% for transrectal and transperineal prostate biopsies respectively, p = 0387. We note that thresholds

Table 1 - Characteristics of the study cohort according to the utilized prostate biopsy route.

Characteristic	Route of prostate biopsy		p Value
	Transrectal	Transperineal	
Number of men, n (%)	2,295 (70.3)	969 (29.7)	-
Median age, years (IQR)	68 (62-73)	68 (62-74)	0.556
Median serum PSA, ng/mL (IQR)	7.1 (5.2-11)	7.4 (5.4-10.7)	0.294
Abnormal DRE, n (%)	132 (25.7)	68 (26.0)	0.214
Median prostate volume, mL (IQR)	55 (40-79)	54 (39-76)	0.178
Prior negative prostate biopsy, n (%)	690 (30.1)	273 (28.1)	0.294
Family history of PCa, n (%)	121 (5.3%)	67 (6.9)	0.231
PI-RADS, n (%)			
1-2	230 (10.0)	83 (8.6)	0.427
3	565 (24.6)	200 (20.6)	0.235
4	987 (43.0)	459 (47.4)	0.189
5	513 (22.4)	227 (23.4)	0.201
Overall PCa detection, n (%)	1,315 (57.3)	651 (67.2)	0.001
csPCa, n (%)	940 (41.0)	445 (45.9)	0.016
iPCa, n (%)	375 (16.3)	169 (17.2)	0.441

IQR = interquartile range; n = number; PI-RADS = prostate imaging-reporting and data system; PCa = prostate cancer; csPCa = clinically significant PCa; iPCa = insignificant PCa.

Figure 1 - Calibration curves of the BCN-MRI PM in transrectal prostate biopsies (A) and transperineal prostate biopsies (B).

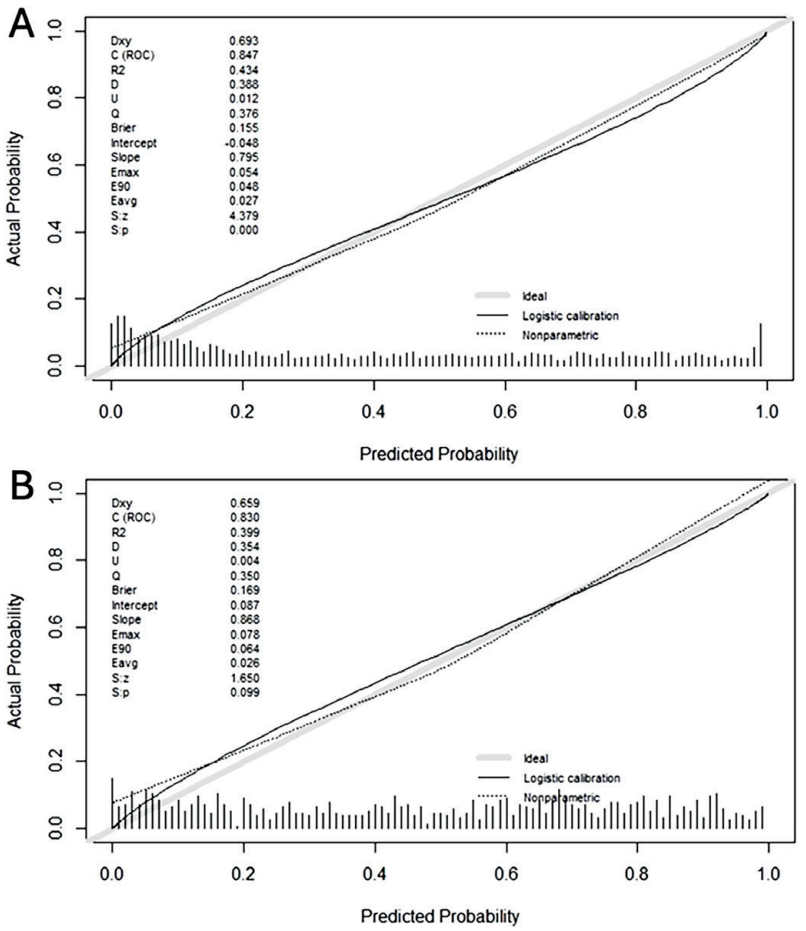
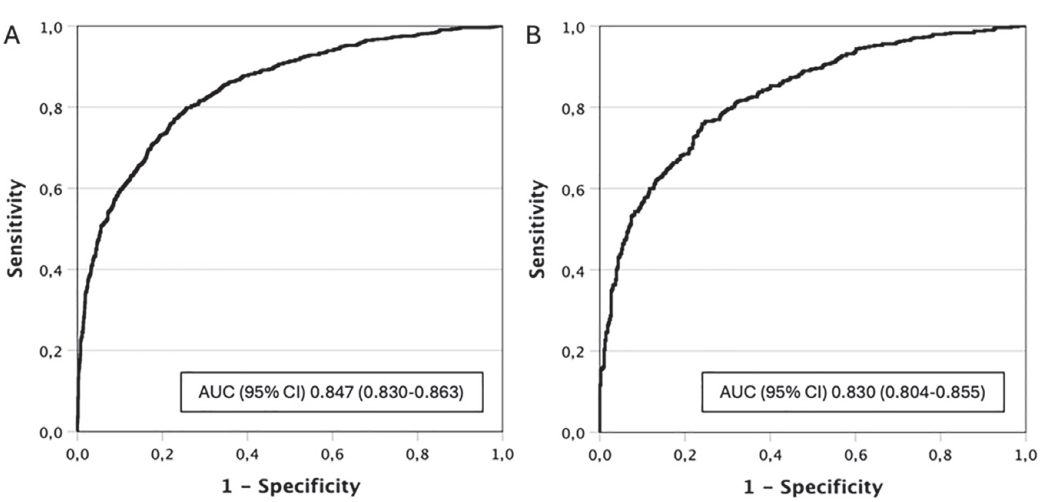


Figure 2 - Discrimination ability of the BCN-MRI PM for csPCa detection in transrectal prostate biopsies (A) and transperineal prostate biopsies (B



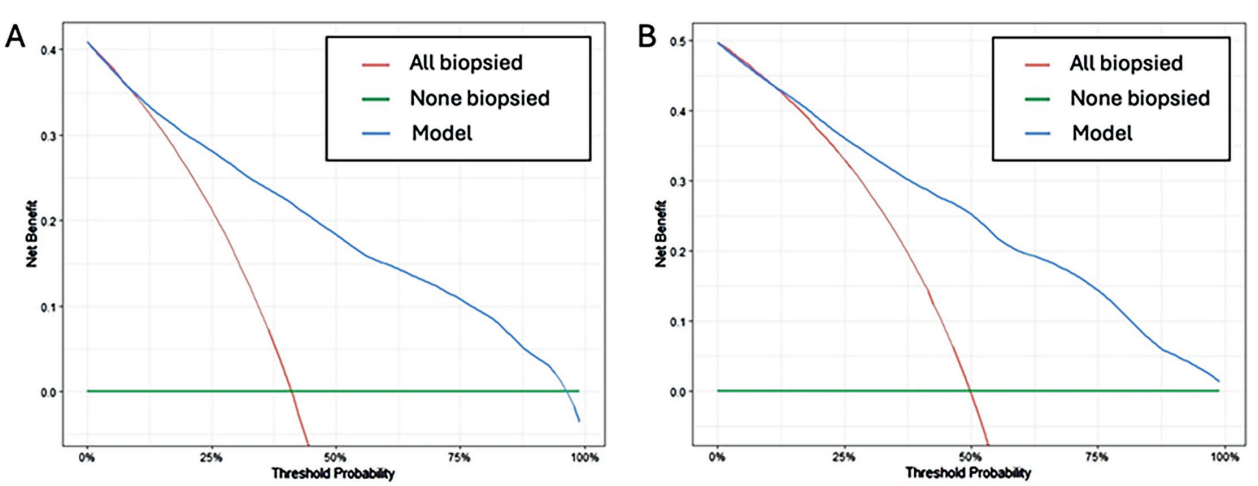
of the BCN-MRI PM were higher when the transperineal route was used. The avoided prostate biopsies with 100% sensitivity of the BCN-MRI PM were 17 (0.7%) for transrectal biopsies and 9 (0.9%) for transperineal biopsies (p =0.438). For csPCa 97.5% sensitivity, they were 344 (15%) and 112 (12.6%), respectively, (p =0.395). For csPCa 95% sensitivity, they were 557 (24.3%) and 229 (22.6%), respectively, (p =0.387).

DCAs showed a similar net benefit of the BCN-MRI PM over biopsying all men with both prostate biopsy routes beginning at lower than 10% and 15% threshold probabilities of csPCa for transrectal and transperineal prostate biopsies, respectively, Figure-3 A-B.

DISCUSSION

The BCN-MRI PM is based on PI-RADS score and some clinical variables that resulted independent for csPCa prediction in prostate biopsies. PSA density was the most weighed independent predictor after MRI (16, 17), that was expressed as serum PSA and MRI derived from prostate volume to avoid the manual calculation of PSA density. The BCN-MRI PM has been satisfactorily validated in men suspected of having PCa undergoing pre-biopsy mpMRI reported with the PI-RADS v2.1, and those undergoing prostate biopsies conducted via the transrectal and transperineal routes. This is important, since the BCN-MRI PM has been developed and validated using the PI-RADS v2.0 and transrectal biopsies. This successful validation guaranties the accuracy of the BCN-MRI PM predictions when PI-RADS v2.1 and transrectal biopsies are employed. The scheme of prostate biopsy conducted in this external validation study has been the same employed in the development cohort of the BCN-MRI PM, which obtained 2- to 4-core MRI-TRUS fusion-targeted biopsies of PI-RADS lesions ≥ 3 and/or 12-core systematic biopsy when the PI-RADS was <3, although some current reports suggest that systematic biopsies can be reduced (18), even to biopsy only the index lesion using a mapping scheme (19). This external validation has been successful despite the improved csPCa detection observed when the transperineal route is employed to conduct prostate biopsies. We noted that transperineal prostate biopsies detected 45.9% of csPCa while 41.7% when transrectal route was used. This finding has been previously reported, especially in anterior and apical suspicious lesions (20, 21). Similar differences in csPCa detection rates were observed in the first external validation conducted in the Barcelona metropolitan area, due to differences between the baseline characteristics of the development and validation cohorts, showing the good performance of the BCN-MRI PM (8). The risk threshold for predicting the same sensitivity for csPCa detection was higher in men

Figure 3 - Net benefit of BCN-MRI PM application over biopsying all men through transrectal route (A) and transperineal route (B).



who underwent transperineal prostate biopsies than in those who underwent the transrectal route. The novelty of selecting the appropriate threshold in the BCN-MRI risk calculator to select candidates for prostate biopsy is thus very useful when csPCa individualized predictions are assessed against each prostate biopsy route (8).

External validations of predictive models are necessary before employing them in new populations with different characteristics than those observed in the development cohort, and are frequently needed to perform recalibrations or adjustments of the thresholds for assessing accurate predictions (22-26). Validations are also necessary when changes in diagnostic approach occur in the same population where the predictive model was developed or in the outcome variables (27).

The European Association of Urology currently suggests the design of risk-stratified pathways using predictive models with the objective of reducing the demand for MRI exams and selecting more appropriate candidates for prostate biopsy, while also reducing the over-detection of iPCa. This is the next step in improving the current diagnostic approach for the early detection of csPCa (28). This was the reason for developing and validating the currently named BCN-predictive model 1, which is applied before the MRI exam, using the age, PCa family history, type of prostate biopsy (initial vs. repeated), DRE (normal vs. suspicious), and DRE-prostate volume category (29). Incorporation of DRE-prostate volume category was due to the importance of prostate volume as a csPCa predictive variable, since TRUS is not currently used with only this aim (30). The corresponding BCN-risk-calculator 1 is available at the same website as the BCN-MRI risk calculator, now named the BCN-risk calculator 2. Using both BCN-risk calculators, after an initial stratification based on the serum PSA level and DRE characteristic (31), we have designed a risk-organized pathway reducing MRI exams and prostate biopsies by more than a quarter with lower loss of csPCa than the currently recommended strategy of avoiding prostate biopsies in men with PI-RADS <3 (32-34). This risk-organized pathway is more efficient than that proposed by Remmers et al., based on sequential application of the Rotterdam-risk calculator 3, and the Rotterdam-MRI risk calculator (35).

Limitations of our validation study include the use of a csPCa definition in prostate biopsies which frequently results in upgrades when the entire prostate gland is analyzed. Multicentricity of the study could produce some lack of homogeneity between both series, and probably differences in quality of MRI exams (36). Additionally, inherent limitations to the predictive models developed with the binary logistic regression algorithm exist. The BCN-MRI PM, developed from a binary logistic regression, reflects the probability of csPCa based on the specific cohort characteristics and diagnostic approach at the time of its development. Changes arising in the same development population as in others where the predictive model will be applied need validations, justifying future recalibrations and adjustments of risk thresholds to ensure accurate predictions.

The real-time updating of classically developed predictive models is a current challenge (37). Dynamic training of predictive models developed with machine learning algorithms, in the setting of federated networks, has the potential to result in continuous validated risk calculators at each partner site, ensuring accurate and lasting predictions across multiple locations (38).

CONCLUSIONS

The BCN-MRI PM has been successfully validated in men suspected of having PCa who undergo MRI exams reported with PI-RADS v2.1, and transperineal prostate biopsies. This study examined data from the csPCa early detection program of Catalonia, a region of 7.9 million inhabitants.

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CONFLICT OF INTEREST

None declared.

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Correspondence address:
Juan Morote, MD, PhD
Depart. of Urology, Vall d´Hebron University Hospital
Po Vall d´Hebron 119-129, Barcelona 08035, Spain
Telephone: +34 629 011 936
E-mail: juan.morote@uab.cat

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A complete year of urology residency training under COVID-19: impact on education and health

João Victor T. Henriques [1], José A. Prezotti [1], Karin M. Anzolch [2], Gustavo Ruschi [3], Gilberto Almeida [4], Leonardo Seligra [5], Luciano A. Favorito [6], Alfredo F. Canalini [7], Roni de C. Fernandes [8], Fransber R. A. Rodrigues [9], Caroline Santos Silva [10], Anna Sophia Candiotto Pereira [11], José de Bessa Jr. [10], Cristiano M. Gomes [1]

[1] Divisão de Urologia, Faculdade de Medicina da Universidade de São Paulo - FMUSP, São Paulo, SP, Brasil; [2] Serviço de Urologia, Hospital Moinhos de Vento, Porto Alegre, RS, Brasil; [3] Universidade Federal do Espírito Santo – UFES, Vitória, ES, Brasil; [4] Universidade do Vale do Itajaí – UNIVALE, Itajaí, SC, Brasil; [5] Disciplina de Urologia, Universidade Federal do ABC - UFABC, Santo André, SP, Brasil; [6] Unidade de Pesquisa Urogenital, Universidade do Estado do Rio de Janeiro - UERJ, Rio de Janeiro, Rio de Janeiro, RJ, Brasil; [7] Disciplina de Urologia, Universidade do Estado do Rio de Janeiro - UERJ, Rio de Janeiro, Rio de Janeiro, RJ, Brasil; [8] Faculdade de Ciências Médicas, Santa Casa de São Paulo, São Paulo, SP, Brasil; [9] Divisão de Urologia, Universidade de Brasília - UNB, Brasília, DF, Brasil; [10] Departamento de Cirurgia, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brasil; [11] Núcleo Técnico. Instituto de Pesquisa, Gestão e Tecnologia – INTEC. Belo Horizonte, MG, Brasil

ABSTRACT

Objectives: To evaluate the impact of COVID-19 pandemics on clinical and surgical practice, educational activities, health and lifestyle behavior of Brazilian urology residents after 1 year of socio-economic restrictions.

Materials and Methods: An electronic survey was e-mailed to all postgraduate (PG) students registered by the Brazilian Society of Urology. The survey included an assessment of socio-demographic, clinical practice, educational, health-related and behavior parameters. We also evaluated which subareas of urology were predominantly affected. A similar survey was adapted and sent to the directors of all urology residency programs.

Results: COVID-19 pandemic has severely impacted the clinical, surgical, and educational activities of urology residents in Brazil. Urology residents reported >50% decrease in multiple surgical modalities. We highlight kidney transplantation surgeries (66.2%), minor surgeries (62.3%), endoscopic surgeries (42.6%) and reconstructive surgeries (38.8%). This could represent a critical skills gap that residents may face beyond the COVID-19 pandemic. Furthermore, PG students faced stressful situations that caused worsening of mental and physical health, such as getting redirected to assistance of COVID-19 patients (66.9%), and high rate of infection by SARS-CoV-2 (58.2%).

Conclusions: The COVID-19 pandemic has severely impacted the clinical, surgical, and educational activities of urology residents in Brazil. This could represent a critical skills gap that residents may face beyond the COVID-19 pandemic. PG students faced stressful situations that caused worsening of mental and physical health such as redirection to assistance of COVID-19 patients, concern about their own contamination and of family members.

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João Victor Henriques
https://orcid.org/0000-0002-5552-4982

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INTRODUCTION

The emergence of the COVID-19 pandemics transformed the medical assistance all around the World. It led to a great reduction of medical consultations, diagnostic evaluations and surgeries of any kind (1, 2). While the pandemics has subsided, it has left enduring sequelae in the health system and profound impacts on medical training (3, 4).

Urology residents have dealt with major challenges not only in terms of medical training, but also regarding their personal lives, health and well-being (5). There was a great decrease in hands-on urological activities such as elective consultations and surgeries, as well as educational activities such as general meetings, classes and journal club discussion (6, 7). In addition, social distancing has caused changes in the residents' lifestyle. Many have been experiencing anxiety disorders and feeling of exhaustion (8-10). Compensation for educational damage has been inconsistent and there is a need to understand the real magnitude of this damage and other impacts on medical residence. Most studies on this subject evaluated the short-time impact of COVID-19 and the long-term impact remains unknown.

In Brazil, COVID-19 started in March/2020, which coincided with the start of the new residency year. In this study, we evaluated the impact of COVID-19 on a complete year of urology residency training. Our main hypothesis is that the COVID-19 pandemic had a profound impact on the training of urology residents due to the decrease in surgical procedures and theoretical educational activities. Furthermore, we believe that this sub-group of healthcare professionals had higher rates of SARS-CoV-2 infection than their peers and significant harm to mental health. The primary goal was to evaluate clinical practice and urological training during the whole year of residency they had just completed under the COVID-19 pandemics (March/2020 to February/2021). Secondary goals involved assessing residents` infection rate and health parameters. We also evaluated the opinion of the directors of urology programs on these topics.

MATERIALS AND METHODS

An electronic survey was e-mailed in June/08/2021 to all postgraduate students (PGY 3 to 5) from official urology residency programs (URP) registered by the Brazilian Society of Urology, during the academic year starting in March/2020. Data collection was closed on July/04/2021. The invitation e-mail contained a link to a 46-question, web-based survey (Supplementary material 1, see more). All questions were closed-ended, multiple choice. The survey included an assessment of socio-demographic, clinical practice, educational, health-related and behavior parameters. A similar survey was adapted and sent to the directors of all URPs. The invitation e-mail contained a link to a 27-question web-based inquiry (Supplementary material 2, see more). The questionnaire addressed many of the same points evaluated by the residents, from the perspective of the program director.

Volume of medical activities and impact on different urological subareas: We assessed the volume of consultations, exams and surgical procedures, but also the resident's perception of prejudice on their training in each urological sub-area. We also assessed urology resident's deployment to the front-line treatment of COVID-19 patients and the availability of personal protective equipment.

Impact on educational activities: We evaluated the impact of the pandemics on in-person educational activities, such as classes, clinical meetings and grand round discussions and evaluated the new formats of online urological training implemented by the residency programs. Residents were asked about their preferences regarding online urological education and additional training offered by their residency program to compensate for the disturbed education.

Impact of COVID-19 pandemics on urology residents' health: We investigated the rate of SARS-CoV-2 infection among urology residents and the severity of the disease. We also evaluated health parameters and lifestyle changes during the studied period, including weight gain, physical activities, alcoholic beverages consumption, sexual activity, satisfaction with general health, depressive symptoms and feeling of exhaustion.

Data collection and Statistical analyses

Data were initially elaborated using Survey Monkey® software online. Quantitative variables were expressed as medians and interquartile ranges, while qualitative variables were expressed as absolute values, percentages, or proportions.

Student´s t or ANOVA was used to compare continuous variables. Categorical variables were compared using the Chi-squared or Fisher's exact test. Associations were described as Odds Ratios with respective confidence intervals. All tests were 2-sided and a p value < 0.05 was considered statistically significant. GraphPad Prism, version 8.0.4, San Diego-CA, USA, was used for data analysis.

RESULTS

A total of 157 urology residents completed the survey, representing 33.5% of all the residents in the country. Most respondents (89.1%) were men, and the median age was 31 (± 3) years. Participants were 37 (23.5%) PGY-3 residents, 55 (35.0%) PGY-4 and 65 (41.4%) PGY-5 residents. The distribution of participants was proportional to the actual distribution of Brazilian urology residents across the country's five geographic regions. São Paulo State accounted for 37.1% of participants.

Most participants (82.8%) attend a URP in a public hospital and most respondents (82.0%) stated that their hospital was transformed in a referral center for COVID-19 patients, with a very high volume of admissions throughout most of the 12-month period of the study.

Impact on medical activities of different urological subareas

Table-1 shows the impact of one year of COVID-19 on the volume of various urological clinical and surgical activities in comparison to the pre-pandemic year. All activities were significantly reduced. A reduction of >50% was reported by most participants in kidney transplant surgery (66.2%); minor surgeries (62.3%) (i.e., vasectomy, circumcision, hydrocelectomy) and urodynamic testing (53.4%). Areas that were least affected

Table 1 - Impact of one year of COVID-19 on urology residents' practice.

Practice activity	%
Elective patient visits	
Remained stable	10.8
Decreased up to 25%	21.6
Decreased 25 to 50%	31.2
Decreased 50 to 75%	25.4
Decreased > 75%	10.8
Emergency patient visits	
Remained stable	33.1
Decreased up to 25%	26.7
Decreased 25 to 50%	19.7
Decreased 50 to 75%	15.9
Decreased > 75%	4.4
Minor surgeries (i.e. vasectomy, circumcision, hydrocelectomy)	
Remained stable	5.7
Decreased up to 25%	12.7
Decreased 25 to 50%	19.1
Decreased 50 to 75%	22.9
Decreased > 75%	39.4
Endoscopic surgeries (i.e. TURP*, TURB)**	
Remained stable	7.6
Decreased up to 25%	20.3
Decreased 25 to 50%	29.3
Decreased 50 to 75%	25.4
Decreased > 75%	17.2
Endoscopic lithiasis surgery (i.e. ureterolithotripsy)	
Remained stable	26.7
Decreased up to 25%	23.5
Decreased 25 to 50%	21.0
Decreased 50 to 75%	15.2
Decreased > 75%	13.3

Major oncologic surgeries	
Remained stable	24.8
Decreased up to 25%	22.9
Decreased 25 to 50%	26.7
Decreased 50 to 75%	14.6
Decreased > 75%	10.8
Reconstructive surgeries (38.8%)	
Remained stable	24.8
Decreased up to 25%	11.4
Decreased 25 to 50%	24.8
Decreased 50 to 75%	19.1
Decreased > 75%	19.7
Kidney Transplants	
Remained stable	7.0
Decreased up to 25%	8.2
Decreased 25 to 50%	18.4
Decreased 50 to 75%	24.8
Decreased > 75%	41.4
Diagnostic procedures (Cystoscopies)	
Remained stable	18.5
Decreased up to 25%	18.5
Decreased 25 to 50%	32.6
Decreased 50 to 75%	18.5
Decreased > 75%	11.5
Urodynamic Testing	
Remained stable	10.8
Decreased up to 25%	18.4
Decreased 25 to 50%	17.2
Decreased 50 to 75%	19.7
Decreased > 75%	33.7

* TURP: Transurethral resection of prostate; ** TURB: Transurethral resection of bladder tumor

included emergency consultations (20.3%), major oncologic surgeries (25.4%), and endoscopic surgeries for lithiasis (28.5%).

Figure-1 depicts the resident's perception of prejudice on their training in each urological subspecialty assessed with a visual analog scale ranging from 0 to 10 (0 being the least prejudice). Uro-oncology was the least affected subspecialty (4.4 ± 2.9), followed by lithiasis (4.8 ± 2.8). The areas considered with worst prejudice on training were sexual medicine/andrology (6.9 ± 2.9) and female urology/neuro-urology (6.7 ± 2.7).

PGY-5 were considered, by all groups combined, as those with the greatest educational damage (47.7%). Interestingly, however, PGY-5 signaled that the most harmed group was PGY-4.

Most residents (66.9%) were relocated to work in the front-line treatment of COVID-19 patients, at some point, during the evaluated period. Regarding the availability of personal protective equipment (PPE): 53 (47.7%) reported shortage of N-95 masks and 51 (45.9%) of waterproof aprons.

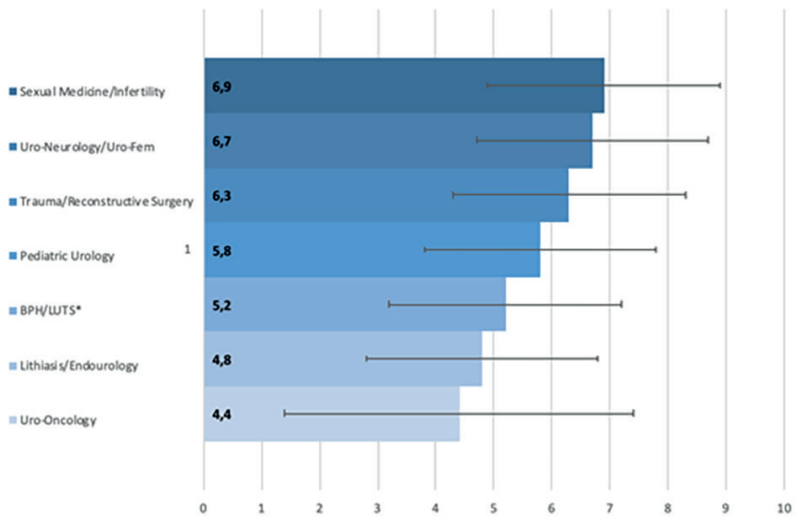
Impact on educational activities

Many scientific and educational activities were cancelled during this period. Bedside clinical rounds (49.0%) and urology department meetings (46.5%) were the two activities that were more frequently cancelled in the period. Only 28 (17.8%) residents claimed that their URP did not cancel any activity. Several smart learning modalities and online meetings and contents were developed.

In our cohort, 116 (73.9%) and 95 (60.5%) urology residents mentioned the general urology department meeting and clinical cases discussion as the most implemented online tools by their URP. Furthermore, 119 (76.3%) urology residents attended regular webinars focused on clinical cases and journal clubs; and 94 (60.2%) watched on-line lectures.

Regarding the intention to supplement urological training after the end of the residency program, 40 (61.5%) PGY-5 declared they would like to pursue fellowships in some subspecialty area and 28 (43.0%) would like to take short training periods in focused sub-areas. Only 6 (9.2%) stated they had no interest in further training.

Figure 1 - Resident`s perception of adverse effects in the training of different urological subspecialties (higher scores indicate worse prejudice).



*BPH/LUTS: Benign prostatic hyperplasia/ Lower urinary tract symptoms.

Scale 0 to 10 – zero being the least affected and ten, the most affected on training.

Most residency programs (89.0%) did not offer alternatives to supplement urology training after the end of the year. As a consequence, most residents (74.2%) were mostly dissatisfied with the lack of actions proposed by the program directors.

Impact of COVID-19 pandemics on urology residents' health

Ninety-two (58.6%) respondents claimed to have had COVID-19 infection in the studied period, including 45.9% with unequivocal laboratory confirmation and 12.7% with a clinical diagnosis. Clinical presentation was mild or moderate in all cases with only one respondent reporting the need for hospitalization for a few days. The impact of COVID-19 on urology residents' health parameters is shown in Table-2. Most residents considered themselves satisfied (41.9%) or very satisfied (11.6%) with their general health, while 25.7% were unsatisfied. Sadness or depressive feelings were reported as usual by 22.3% of the participants while exhaustion was reported by 48.7%. The comparison between residents of different years did not result in differences regarding health-related parameters.

Impact of COVID-19 according to the urology residency directors

A total of 58 URP directors completed the survey, representing 74.3% of all programs in the country. They confirmed major reductions of various urological clinical and surgical activities in comparison to the pre-pandemic year.

The magnitude of reduction estimated by directors was similar to the residents' perceptions for elective and emergency consultations, urodynamics, cystoscopies, minor surgeries and endoscopic prostate surgeries. However, the directors diverged from the residents and estimated a lesser degree of reduction of ureteroscopies, oncologic surgeries, reconstructive surgeries and kidney transplantation (Table-3).

Directors` perception of residents training damage in pediatric urology, BPH/LUTS, sexual medicine/infertility and neurourology was similar to the residents` perception. Directors estimated a lesser degree of training harm in uro-oncology and lithiasis/endourology (Table-4). Among URP directors, 45.0% considered the PGY-4 as the most impacted trainees, 31.7% the PGY-5, and 23.3% the PGY-3.

Table 2 - Changes in urology residents' health parameters during the first year of COVID-19.

Health parameters	%
Weight	
Reduced	22.3
Stable	31.8
Increased	45.9
Physical activity	
Reduced	58.6
Stable	19.1
Increased	22.3
Alcoholic beverages intake*	
Reduced	9.2
Stable	53.2
Increased	37.6
Sexual activity	
Is worse	18.6
Is stable	64.7
In better	16.7
Satisfaction with own general health	
Very unsatisfied	11.6
Unsatisfied	14.1
Not satisfied nor unsatisfied	20.6
Satisfied	41.9
Very satisfied	11.6
Frequency of sadness or depressive feelings	
Never	8.9
Rarely	36.9
Sometimes	31.9
Usually	16.6
Very usually	5.7
Feeling of exhaustion	
Never	2.5
Rarely	15.5
Sometimes	33.3
Usually	32.0
Very usually	16.7

*Participants who reported not drinking alcoholic beverages were removed from calculations

DISCUSSION

This study showed the heavy impact of the pandemics` restrictions on urology residents' education and clinical practice. We observed a great decrease in the volume of outpatient visits and elective surgeries. In addition, residents reported negative consequences for their health and well-being, with a great proportion reporting weight gain (45.9%), reduction of physical activities (58.6%), and increasing alcohol intake (37.6%). Mental health was an important issue as well, feeling of sadness or depression (22.3%) and feeling of exhaustion (48.7%) were present in a considerable proportion of respondents. Remarkably, 58.6% of the residents contracted COVID-19.

The study took place in June 2021, 15 months after the onset of the pandemic, with participants commenting on the period from March 2020 to March 2021. This time frame marked the first year of the pandemic, characterized by strict social and economic restrictions. Brazil was particularly hard-hit, ranking second in deaths and third in infections due to COVID-19 throughout this period (11).

In this study, we had 157 participants, representing 33.5% of the 468 eligible urology residents. We were hoping to have a higher participation rate. A previous study assessing the short-term impact of the pandemic on Brazilian urology residents achieved a 58.7% response rate (10). We believe that the physical and emotional fatigue associated with the pandemics restrictions and the fact that the questionnaire was long may have contributed to the lower participation rate in this survey. However, our participation rate aligns other similar surveys, ranging from 15% to 60.8% (5, 9, 12). Most participants were PGY-5 residents (41.40%), followed by PGY-4 (35.03%) and PGY-3 (23.57%) residents. We hypothesize that senior residents were more inclined to participate due to heightened concerns about the pandemic's impact on their training.

Residents reported a substantial reduction in all clinical and surgical activities. Similar results have been observed globally. In Turkey, an online survey assessed the impact of the pandemics on functional urology practice, and found a decrease in outpatient clinics, urody-

Table 3 - Comparison between residents and directors regarding the decrease in the volume of surgeries and consultations in URPs.

	RESIDENTS		DIRECTORS		
	< 50% reduction	> 50% reduction	< 50% reduction	> 50% reduction	P VALUE
Elective Consultations	100	57	45	15	0.146
Emergency Consultations	125	32	53	08	0.417
Urodynamics	73	84	27	34	0.879
Cystoscopies	109	48	38	22	0.419
Minor Surgeries	59	98	24	36	0.757
TURP*	90	67	41	19	0.163
Ureteroscopies	112	45	52	09	0.036
Oncologic Surgeries	117	40	53	05	<0.001
Reconstructive Surgeries	53	104	29	27	<0.001
Kidney Transplants	96	61	28	23	<0.001

*TURP: transurethral resection of the prostate.

Table 4 - Comparison between residents and directors regarding their evaluation of harm in urologic training in different sub-areas.

	RESIDENTS	DIRECTORS	
	Mean	Mean	P VALUE
Lithiasis/Endourology	**4.8 (± 2.8)**	**3.5 (± 2.3)**	**0.003**
BPH/LUTS*	5.2 (± 2.9)	5.0 (± 2.3)	0.654
Sexual Medicine/Infertility	6.9 (± 2.9)	6.8 (± 2.3)	0.517
Trauma/Reconstructive Surgery	6.3 (± 2.9)	5.8 (± 2.4)	0.255
Uro-Oncology	**4.4 (± 3.0)**	**3.2 (± 2.0)**	**0.013**
Uro-Neurology/Uro-Fem	6.7 (± 2.7)	6.5 (± 2.2)	0.280
Pediatric Urology	5.8 (± 3.3)	6.1 (± 2.8)	0.636

*BPH/LUTS: Benign prostatic hyperplasia/ Lower urinary tract symptoms.
Scale 0 to 10 – zero being the least prejudice and ten, the most prejudice.

namic testing and elective surgeries (13). Comparable findings were reported in Italy (14), aligning with urological society guidelines that recommended prioritizing more urgent diseases, the postponement of which could affect cure chances (15, 16).

The pandemic led to the cancellation of most educational and scientific activities. Most of the residents made use online smart learning tools, such as discussion-focused webinars (76.3%) and pre-recorded on-line lectures (60.2%). These findings are in line with other countries. In Italy, 38.8% of urology residents utilized webinars for smart learning (9). In Indonesia, web-based video conference was the most used method educational activity during the pandemic (16). A survey by the Ameri-

can Confederation of Urology (CAU) showed that 93% of residents attended webinars during the pandemic (17).

COVID-19 restrictions have impacted people's lifestyles around the World. There were reports of decreased physical activity, weight gain, and increased alcohol and tobacco consumption (18, 19). Brazilian physicians reported similar effects (4, 10). In the present study, 58.6% of the residents reported reduced physical activity, and 45.8% reported weight gain throughout the one-year study period. Over 25% of the participants were dissatisfied with their general health. Additionally, 48.7% had a feeling of exhaustion and 21.4% reported recurrent sadness and depressive feelings. These findings indicate a significant impact on overall well-being and mental health which is very concerning. Residents from different PGYs were similarly affected. Our findings align with other studies reporting mental health problem rates ranging from 33 to 57.6% (20, 21). A systematic review of 33,062 frontline health care workers, found a pooled prevalence rate of anxiety of 23.2%, and depression prevalence of 23.8% (22). Burnout among physicians is strongly associated with the career disengagement, suboptimal patient care and patient safety incidents (23).

Our findings indicate that 58.6% urology residents contracted COVID-19, which is an exceptionally high infection rate among Brazilian urology residents during the pandemic's first year. Contrastingly, only 4.71% of the Brazilian general population were infected with SARS-COV-2 at the same period (11). Globally, COVID-19 rates in medical residents varied from 5.0% to 26.3% at different times.

In our study, 105 (66.8%) residents were redeployed to work with patients infected with COVID-19. In Spain, 50% of urology residents were recruited to COVID-19 specific units (24). In the United States, urology program directors reported that 26% of residents were shifted to treat COVID-19 patients (5). A key concern regarding our residents was workplace exposure and appropriate PPE availability. Nearly half of the participants reported a lack of N-95 masks (47.7%), waterproof aprons (45.9%), protective goggles (42%) at their hospitals. Comparable outcomes were noted in New York, where the practice of mask reuse was documented (25), and in France, where 43% of residents reported inadequate access to PPE (19).

Most (93%) PGY-5 residents expressed an intention to complement their training after finishing urology residency. US studies showed significant concern among residents and URP directors regarding the impact of COVID-19 in medical training, including failure to meet clinical visit and surgery goals and fearing a lack of skill for a fellowship or future job (26, 27). Most URPs did not present a plan to mitigate the training damage. Despite the uniformity of prejudice in urology training across multiple countries, there were no effective compensatory strategies (9, 28).

This study's primary strength lies in its evaluation of urology residents after one year of the pandemic, coinciding with a full year of residency. Participants were evenly distributed across various postgraduate levels and represented all five geographic regions of the country. We conducted a comprehensive assessment of the pandemic's impact on a full year of urology residency training, covering medical practice, educational activities, and health and lifestyle parameters. Additionally, we identified which subareas of urology were predominantly affected based on surgery volume and residents' perceptions of prejudice. The study provides insight into URP directors' views on resident training. A notable limitation of the study is the length of the questionnaire, which may cause participants to become bored while completing it. Additionally, many of the instruments used to evaluate various parameters are not validated. For example, mental health was assessed with a single question instead of using a validated questionnaire. Another limitation is the requirement for participants to compare their current state with the previous year, which could introduce recall bias. Further research should confirm these findings across different medical specialties to develop strategies for mitigating training losses. This study sheds light on the challenges faced by urology postgraduate students during the pandemic.

CONCLUSION

The COVID-19 pandemic has severely impacted the clinical, surgical, and educational activities of urol-

ogy residents in Brazil, regardless of the residency year. PG students faced stressful situations that caused worsening of mental and physical health such as redirection to assistance of COVID-19 patients, concern about their own contamination and of family members and shortages in PPE, in addition of the aforementioned educational loss. This could represent a critical skills gap that residents' may face beyond the COVID-19 pandemic. The program directors and entities responsible for the quality of medical training must assess the difficulties imposed by the pandemic and formulate a compensation plan to try to soften the impact on training residents. Future research, with a longer follow-up time will be needed to accurately measure the impact of this pandemic on urology training.

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STATEMENTS RELATING TO OUR ETHICS AND INTEGRITY POLICIES:

- Data availability statement: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
- Ethics of approval statement: The study was approved by the local ethics committee (Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo – CAPPesq - approval number 13029).
- Patient consent statement: All human subjects provided written informed consent with guarantees of confidentiality
- Clinical trial registration: this is not an interventional clinical trial, this is an epidemiological study.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
João Victor Teixeira Henriques, MD
Divisão de Clínica Urológica
Hospital das Clínicas da Universidade de São Paulo
Av. Dr. Enéas de Carvalho Aguiar, 255 / 710F 7º Andar
São Paulo, SP, 05403-000, Brasil
Telephone: +55 11 2661-8080
E-mail: joao.victor.th@gmail.com

ORIGINAL ARTICLE

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Transperineal versus Transrectal MRI/TRUS fusion-guided prostate biopsy in a large, ethnically diverse, and multiracial cohort

Lorenzo Storino Ramacciotti [1, 2], David Strauss [1, 2], Francesco Cei [1], Masatomo Kaneko [1, 2], Daniel Mokhtar [1], Jie Cai [1], Delara Jadvar [1, 2], Giovanni E. Cacciamani [1, 3], Manju Aron [1, 4], Pierre B. Halteh [3], Vinay Duddalwar [1, 3], Inderbir Gill [1], Andre Luis Abreu [1, 2, 3]

[1] USC Institute of Urology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; [2] Center for Image-Guided Surgery, Focal Therapy and Artificial Intelligence for Prostate Cancer, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; [3] Department of Radiology Keck School of Medicine, University of Southern California, Los Angeles, California, USA; [4] Department of Pathology Keck School of Medicine, University of Southern California, Los Angeles, California, USA

ABSTRACT

Purpose: To compare transperineal (TP) vs transrectal (TR) magnetic resonance imaging (MRI) and transrectal ultrasound (TRUS) fusion-guided prostate biopsy (PBx) in a large, ethnically diverse and multiracial cohort.

Materials and Methods: Consecutive patients who underwent multiparametric (mp) MRI followed by TP or TR TRUS-fusion guided PBx, were identified from a prospective database (IRB #HS-13-00663). All patients underwent mpMRI followed by 12-14 core systematic PBx. A minimum of two additional target-biopsy cores were taken per PIRADS≥3 lesion. The endpoint was the detection of clinically significant prostate cancer (CSPCa; Grade Group, GG≥2). Statistical significance was defined as $p<0.05$.

Results: A total of 1491 patients met inclusion criteria, with 480 undergoing TP and 1011 TR PBx. Overall, 11% of patients were Asians, 5% African Americans, 14% Hispanic, 14% Others, and 56% White, similar between TP and TR ($p=0.4$). For PIRADS 3-5, the TP PBx CSPCa detection was significantly higher (61% vs 54%, $p=0.03$) than TR PBx, but not for PIRADS 1-2 (13% vs 13%, $p=1.0$). After adjusting for confounders on multivariable analysis, Black race, but not the PBx approach (TP vs TR), was an independent predictor of CSPCa detection. The median maximum cancer core length (11 vs 8mm; $p<0.001$) and percent (80% vs 60%; $p<0.001$) were greater for TP PBx even after adjusting for confounders.

Conclusions: In a large and diverse cohort, Black race, but not the biopsy approach, was an independent predictor for CSPCa detection. TP and TR PBx yielded similar CSPCa detection rates; however the TP PBx was histologically more informative.

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Lorenzo Storino Ramacciotti
https://orcid.org/0000-0002-0615-9720

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INTRODUCTION

An accurate prostate cancer (PCa) diagnosis relies on a quality prostate biopsy (PBx) followed by histological evaluation (1). The European Association of Urology guidelines strongly recommend the transperineal (TP) approach over the transrectal (TR) approach as the gold standard for prostate biopsy. This recommendation is based on observational studies and meta-analyses showing lower rates of infectious complications and hospital re-admissions for sepsis (2, 3). In contrast, the American Urological Association guidelines state that clinicians may choose either a TR or TP biopsy approach (4). One meta-analysis suggested higher PCa and clinically significant PCa (CSPCa) detection rates using the TP approach, especially for anterior lesions (5), while another stated no differences between approaches (6).

Randomized clinical trials comparing both approaches have recently been published, evidencing similar cancer detection and complication rates (7-10). However, these trials' cohorts consisted mostly of white patients, limiting the findings' applicability to other racial and ethnic groups. We hypothesized that PCa detection rates on different PBx approaches might be impacted by the patient's race and ethnicity.

Hence, this study aimed to compare magnetic resonance imaging (MRI) transrectal ultrasound (TRUS) fusion-guided TP and TR PBx cancer detection rates, histologic findings, and periprocedural outcomes in a multiracial and ethnically diverse cohort.

MATERIALS AND METHODS

Study design and population

Consecutive patients who underwent multiparametric (mp) MRI followed by TP or TR TRUS-fusion guided PBx, between January 2016 and May 2023, were identified from a prospective institutional database (IRB #HS-13-00663). Inclusion criteria were: I) patients who underwent mpMRI within 6 months of biopsy: II) patients who underwent MRI/TRUS fusion TP or TR PBx. Exclusion criteria were: I) any prior treatment for PCa; II) any prior prostate surgery; III) saturation biopsies; IV) patients with mpMRI that didn't meet Prostate Imaging Reporting & Data System (PIRADS) standards (11, 12).

MRI acquisition and interpretation

The mpMRIs (T2W, DWI, ADC and DCE) were acquired and interpreted in accordance with the relevant PIRADS version (2.0 or 2.1) (11, 12) prevalent during the biopsy timeframe, as previously described (13-17). Images were interpreted by radiologists with over 5 years of expertise in prostate mpMRI reading. The lesion with the highest PIRADS score, followed by the largest dimension, was defined as the index lesion.

Prostate biopsy protocol

Prostate biopsies were performed transperineally or transrectally by a single urologist (ALA) (Figure-1). All procedures were performed using a three-dimensional organ-tracking elastic image fusion system (Trinity, Koelis®, France) and an 18G needle-biopsy, as previously described (13-19). All patients underwent mpMRI followed by a 12–14 core systematic biopsy (SB), with at least two additional target biopsy (TB) core samples per suspicious lesion for patients with PI-RADS 3-5 lesions (Figure-2). TP and TR PBx were routinely offered and performed under local anesthesia in the outpatient clinic. However, PBx were eventually performed under sedation in the operating room based on patient preference or for the initial TP PBx cases. The PBx specimens were individually labeled and submitted in separate containers for uropathologist evaluation according to the International Society of Urological Pathology (ISUP) guidelines (20).

Empiric antibiotic prophylaxis was prescribed according to American Urological Association recommendations (21). Patients undergoing TR PBx received 3 days of Ciprofloxacin, Bactrim, or Cefuroxime with augmentation of Gentamicin IM at the time of biopsy. Povidone-iodine preparation was not performed before TR or TP PBx. Not all patients undergoing TP PBx received antibiotic prophylaxis (22). When the TP PBx was integrated into our institution's clinical practice, between 2016 and 2017, there was insufficient evidence supporting an antibiotic-free procedure at that time. The initial patients received a single dose of Cefuroxime 500mg (21, 23). However, with the emergence of new level 1 evidence supporting the safety of performing TP PBx without antibiotic prophylaxis, only specific subsets of patients continued to receive antibiotic prophylaxis thereafter. These included patients with cardiac

Figure 1. Prostate biopsy setup and templates. A) MRI/TRUS fusion-guided free-hand transperineal prostate biopsy. In this procedure, the biopsy gun is inserted through a coaxial needle, minimizing multiple punctures through the perineal skin. The patient remains in the supine position during the entire procedure. B) MRI/TRUS fusion-guided transrectal prostate biopsy. The prostate is sampled by inserting the needle through the rectum, with the patient placed in the left lateral decubitus position.

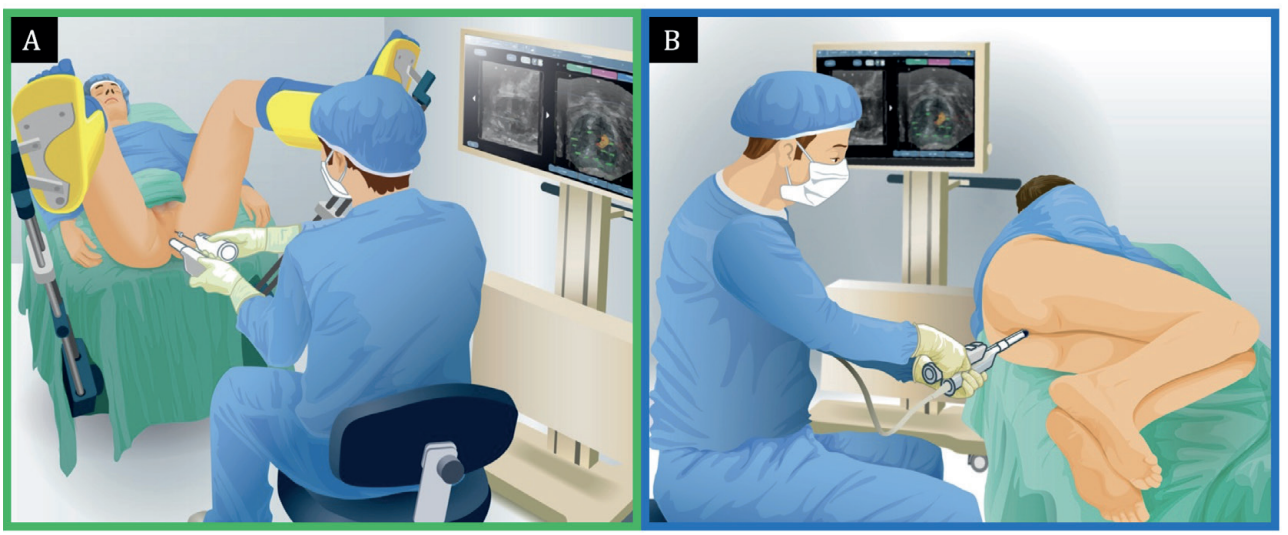
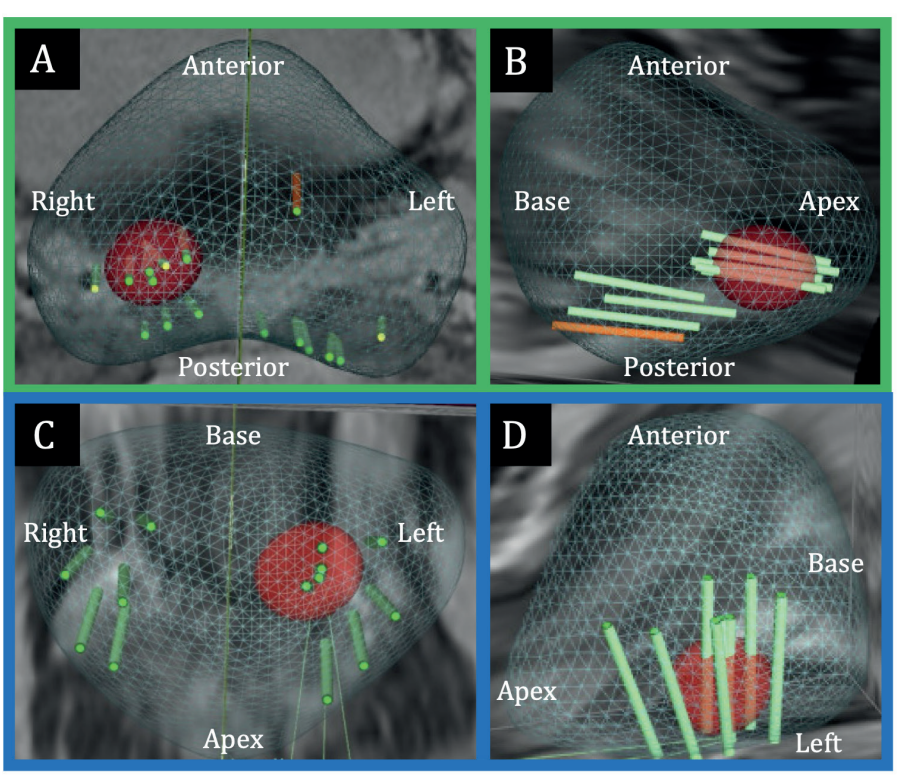


Figure 2. Prostate biopsy templates. A) Axial and B) right sagittal view of the transperineal MRI/TRUS fusion-guided prostate biopsy template. C) Coronal and D) left sagittal view of the transrectal MRI/TRUS fusion-guided prostate biopsy template.



valve disease or replacement, those with a history of acute prostatitis, or those who were immunosuppressed.

Transperineal and transrectal approach

TR approach was offered to all initial patients as the operator did not perform TP PBx in 2016. With the adoption of TP PBx in 2017, patients were offered either the TP or TR approach based on their risk of complications. Specifically, patients with an increased risk of rectal bleeding or urinary tract infections were recommended the TP PBx. Patients with an increased risk of rectal bleeding included those on antiplatelet or anticoagulant therapy or those with a previous history of rectal bleeding on prior PBx. MRI interpretation and lesion location did not define the approach to be used. As of 2023, the operator had transitioned to performing TP PBx, with the TR approach being reserved for cases based on patient's request.

Endpoints and definitions

The primary endpoint was the detection of clinically significant PCa (CSPCa), defined as grade group (GG) ≥ 2 on SB, TB, and SB plus TB. Secondary outcomes included: I) detection of high-grade PCa, defined as GG ≥ 3; II) amount of PCa core length and percent of core involvement by PCa on TB; III) periprocedural outcomes.

The index lesion was defined as the highest PIRADS score, followed by the largest dimension. The location of the index lesion was categorized based on its position in the prostate on MRI: anterior index lesions were defined as those located from the 9:00 to 3:00 o'clock positions, while posterior lesions were located from 3:00 to 9:00 o'clock positions. If the largest lesion spanned both anterior and posterior locations, it was assigned to both locations. This classification was also applied to lesions located at the mid, base, and apex of the prostate. Prostate volume (PV) was estimated based on MRI measurements using the ellipsoid formula (PV = height × width × length × 0.52).

Complications were recorded up to 30 days post-biopsy using the Clavien-Dindo grading system (24, 25). Procedure time was recorded from the instant the ultrasound probe was introduced into the patient's rectum to the moment it was removed. Recorded pain levels were all self-reported immediately after the procedure and exclusive to cases done under local anesthesia (18). Patients were asked to rate on a visual analog scale (0-10) the overall pain experienced throughout the procedure. Baseline demographics and MRI details were also analyzed.

Race and ethnicity were self-reported by patients and defined according to the NIH reporting standards as follows: Hispanic or Latino (Latino), non-Hispanic Asian (Asian), non-Hispanic Black or African American (Black), non-Hispanic White (White), and Other. The Other category includes patients who didn't report or identify as any race or ethnicity, American Indian or Alaska Native, and Native Hawaiian or Other Pacific Islander due to small sample sizes (26).

Statistical Analysis

The statistical software package SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all analyses in this study. Patients were divided into two separate cohorts of PIRADS 1-2 (negative MRI) and PIRADS 3-5 for sub-group analysis. The Wilcoxon rank sum test was used for continuous variables, and Pearson's chi-square was used for categorical variables. Univariable and multivariable logistic and linear regressions were performed to model the dichotomous and continuous outcomes, respectively, to identify clinical parameters related to CSPCa, high-grade PCa detection, and PBx histolologic findings. MRI lesion location was divided into "anterior versus non-anterior lesions" and "apical versus non-apical lesions" for univariable and multivariable analyses. A two-sided p-value of < 0.05 was considered statistically significant.

RESULTS

A total of 1491 patients met the inclusion criteria, with 480 undergoing TP and 1011 TR PBx. Overall, 11% of patients were Asian, 5% Black or African American, 14% Latino, 14% Others, and 56% White, similar between TP and TR (p=0.41). The median age (67 vs 66Y, p=0.048), PSA (6.8 vs 6.5ng/mL, p=0.09), PSA density (0.13 vs 0.12ng/mL2, p=0.27), prostate volume (55 vs 52cc, p=0.53), and PIRADS distribution (PIRADS 3-5, 75% vs 74%, p=0.75) were similar between the TP and TR groups, respectively (Table-1). The median MRI index lesion size (14 vs 12mm, p=0.001) and number of MRI lesions (1 vs 1; p=0.009) were higher for the TP group.

Table 1 - Baseline characteristics of the transperineal and transrectal cohorts.

	MRI								
	All patients			PIRADS 1-2			PIRADS 3-5		
	Perineal	Rectal	p	Perineal	Rectal	p	Perineal	Rectal	p
No. of Patients, n (%)	480	1011		122	265		358	746	
Age, year, median (IQR)	67 (62-72)	66 (61-70)	0.048	65 (60-69)	64 (59-69)	0.3	67 (62-72)	66 (61-71)	0.09
Carlson comorbidity index, median (IQR)	1 (0-2)	1 (0-2)	0.9	0 (0-2)	1 (0-2)	0.5	1 (0-2)	1 (0-2)	0.8
Family History PCa, n (%)	122 (27.5)	261 (27.8)	0.9	37 (33.0)	64 (25.7)	0.16	85 (25.7)	197 (28.5)	0.4
Race, n (%)			0.4			0.07			0.8
White	265 (55.2)	571 (56.5)		62 (50.8)	157 (59.3)		203 (56.7)	414 (55.5)	
Black	25 (5.2)	48 (4.8)		8 (6.6)	18 (6.8)		17 (4.8)	30 (4.0)	
Latino	67 (14.0)	147 (14.5)		19 (15.6)	34 (12.8)		48 (13.4)	113 (15.2)	
Asian	63 (13.1)	101 (10.0)		22 (18.0)	23 (8.7)		41 (11.5)	78 (10.5)	
Other or not reported	60 (12.5)	144 (14.2)		11 (9.0)	33 (12.5)		49 (13.7)	111 (14.9)	
Biopsy History, n (%)			<0.001			0.002			0.001
Naïve	311 (65.6)	524 (51.9)		78 (65.6)	124 (46.8)		233 (65.6)	400 (53.8)	
Negative	91 (19.2)	254 (25.2)		26 (21.9)	75 (28.3)		65 (18.3)	179 (24.1)	
In active surveillance	72 (15.2)	231 (22.9)		15 (12.6)	66 (24.9)		57 (16.1)	165 (22.2)	
PSA, ng/mL, median (IQR)	6.8 (4.9-10.3)	6.5 (4.8-9.7)	0.09	6.9 (5.2-9.9)	6.2 (4.5-8.5)	0.02	6.7 (4.9-10.5)	6.6 (4.9-10)	0.5
PSA density, ng/mL^2, median (IQR)	0.13 (0.08-0.20)	0.12 (0.08-0.20)	0.3	0.11 (0.08-0.17)	0.10 (0.07-0.14)	0.048	0.13 (0.09-0.22)	0.13 (0.08-0.22)	0.9
Suspicion for PCa on DRE, n (%)	113 (23.5)	265 (26.2)	0.3	17 (13.9)	44 (16.6)	0.5	96 (26.8)	221 (29.6)	0.3
Clinical T stage, n (%)*			0.19			0.8			0.2
T1	375 (80.5)	784 (78.6)		111 (94.1)	244 (92.4)		264 (75.9)	540 (73.6)	
T2	62 (27.3)	165 (16.5)		6 (5.1)	18 (6.8)		56 (16.1)	147 (20.0)	
T3/T4	29 (6.2)	49 (4.9)		1 (0.8)	2 (0.8)		28 (8.0)	47 (6.4)	
Prostate Volume, cc, median (IQR)	55 (38-77)	52 (37-75)	0.53	62 (42-86)	59 (43-86)	0.91	52 (37-72)	49 (36-72)	0.37
No. MRI lesions, median (IQR)	1 (1-1)	1 (1-2)	0.23	0 (0)	0 (0)	-	1 (1-2)	1 (1-2)	0.009
MRI index lesion location, n (%)				-	-	-			
Anterior	148 (30.8)	211 (20.9)	<0.001				146 (40.8)	206 (27.6)	<0.001

Posterior	264 (55)	572 (56.6)	0.58	-	-	-	256 (71.5)	561 (75.2)	0.21
MRI index lesion location, n (%)**									
Base	69 (14.4)	159 (15.7)	0.54	-	-	-	65 (18.2)	152 (20.4)	0.42
Mid	190 (39.6)	318 (31.5)	0.002	-	-	-	184 (51.4)	304 (40.8)	0.001
Apex	87 (18.1)	186 (18.4)	0.94	-	-	-	87 (24.3)	179 (24)	0.94
MRI index lesion size, mm, median (IQR)**	14 (10-19)	12 (9-17)	0.001	-	-	-	14 (10-19)	12 (9-17)	0.001
PIRADS score, n (%)			0.75			-			-
PIRADS 1-2	122 (25.4)	265 (26.2)		122 (100)	265 (100)		-	-	
PIRADS 3-5*	358 (74.6)	746 (73.8)		-	-		358 (100)	746 (100)	
PIRADS 3	93 (19.4)	330 (32.6)		-	-		93 (26)	330 (44.2)	
PIRADS 4	139 (29)	271 (26.8)		-	-		139 (38.8)	271 (36.3)	
PIRADS 5	126 (26.3)	145 (14.3)		-	-		126 (35.2)	145 (19.4)	

PIRADS = Prostate Imaging Reporting and Data System; MRI = magnetic resonance imaging; No., number; IQR = Interquartile Range; PCa = prostate cancer; CSPCa = Clinically significant PCa (Grade Group > 1); DRE = digital rectal examination; Anterior lesion on MRI 9-3:00 position; otherwise it's posterior. * DRE findings of a possible clinical stage in case prostate biopsy confirms cancer. ** Index lesion (highest PIRADS, then the largest lesion)

Primary Endpoint

Overall, for all patients, TP detected more CSPCa (48.8% vs 43.1%, p=0.04) compared to the TR PBx on SB plus TB. For PIRADS 3-5, TP detected more CSPCa (60.9% vs 53.9%, p=0.03) on SB plus TB. For PIRADS 1-2, CSPCa detection was not different between biopsy approaches (13.1% vs 12.8%, p=1.0). CSPCa detection rates for anterior (64.2% vs 57.8%, p=0.2) and non-anterior (41.9% vs 39.2%, p=0.4) lesions on MRI were similar between TP and TR PBx, respectively. Detailed biopsy outcomes are reported in Table-2. On a multivariable logistic regression model, age, negative biopsy history, PSA, Black race, suspicious digital rectal examination, PV, PIRADS 3-5, and the number of TB cores taken were independent predictors for CSPCa detection (Table-3). Although the biopsy approach, anterior and non-apical lesions on MRI were independent predictors for CSPCa detection in the univariable analysis, they were not independent predictors in the multivariable analysis.

Secondary outcomes

TP detected more high-grade PCa (27.9% vs 21.7%, p=0.009) in the overall cohort and in the PIRADS 3-5 subgroup (36% vs 28%, p=0.008) on SB plus TB. For PIRADS 1-2, high-grade PCa detection rates (4.1% vs 3.8%, p=0.9) were not different between TP and TR, respectively. On a multivariable logistic regression model for GG≥3 PCa detection, age, PSA, suspicious digital rectal examination, PV, PIRADS 3-5, and non-apical lesions were independent predictors (Supplementary Table-1, see more). The biopsy approach and anterior lesion on MRI were not independent predictors for high-grade PCa detection.

TP PBx had a higher median maximum TB cancer core length (11 vs 8 mm, p<0.001) and percent involvement by cancer (80% vs 60%, p<0.001) than the TR PBx. On multivariable linear regressions, TP PBx was still an independent predictor for higher cancer core length and percent involvement on TB (Supplementary Tables 2 and 3, see more).

Median procedure time was longer for TP PBx (20 vs 19 min, p<0.001) (Supplementary Table-4, see more). The median patient self-reported pain levels were similar between the biopsy approaches (TP 3 vs TR 4, p=0.6). The 30-day complications were low and

Table 2 - Outcomes of transperineal vs transrectal MRI/TRUS fusion prostate biopsy.

	MRI					
	PIRADS 1-2			PIRADS 3-5		
	Perineal	Rectal	p	Perineal	Rectal	p
No. of Patients, n (%)	122	265		358	746	
Prostate Biopsy Pathology						
Grade Group			0.4			
Benign	80 (65.6)	176 (66.4)		101 (28.2)	241 (32.3)	
1	26 (21.3)	55 (20.8)		39 (10.9)	103 (13.8)	
2	11 (9.0)	24 (9.0)		89 (24.9)	193 (25.9)	0.09
3	1 (0.8)	7 (2.6)		52 (14.5)	100 (13.4)	
4	2 (1.6)	2 (0.8)		42 (11.7)	59 (7.9)	
5	2 (1.6)	1 (0.4)		35 (9.8)	50 (6.7)	
No. of TB cores taken, median (IQR)	-	-	-	4 (4-6)	4 (2-5)	<0.001
No. of positive TB cores, median (IQR)	-	-	-	3 (0-5)	1 (0-3)	<0.001
PCa detection SB + TB, N (%)	42 (34.4)	89 (33.6)	0.9	257 (71.8)	505 (67.7)	0.19
PCa detection SB, N (%)	42 (34.4)	89 (33.6)	0.9	208 (58.1)	471 (63.1)	0.11
PCa detection TB, N (%)	-	-	-	233 (65.1)	406 (54.4)	<0.001
CSPCa SB + TB, N (%)	16 (13.1)	34 (12.8)	1.0	218 (60.9)	402 (53.9)	0.03
CSPCa SB, N (%)	16 (13.1)	34 (12.8)	1.0	162 (45.3)	361 (48.4)	0.3
CSPCa TB, N (%)	-	-	-	199 (55.6)	301 (40.4)	<0.001
GG≥3 detection SB + TB, N (%)	5 (4.1)	10 (3.8)	0.9	129 (36)	209 (28)	0.008
GG≥3 detection SB, N (%)	5 (4.1)	10 (3.8)	0.9	89 (24.9)	176 (23.6)	0.6
GG≥3 detection TB, N (%)	-	-	-	116 (32.4)	145 (19.4)	<0.001
CSPCa SB + TB, N (%)						
PIRADS 3	-	-	-	28 (30.1)	104 (31.5)	0.9
PIRADS 4	-	-	-	85 (61.2)	174 (64.2)	0.6
PIRADS 5	-	-	-	105 (83.3)	124 (85.5)	0.7
CSPCa TB per lesion, N (%) PIRADS 3	-	-	-	20 (21.5)	60 (18.2)	0.4
PIRADS 4	-	-	-	80 (57.6)	136 (50.2)	0.17
PIRADS 5	-	-	-	99 (78.6)	105 (72.4)	0.3

GG≥3 SB + TB, N (%)						
PIRADS 3	-	-	-	10 (10.8)	44 (13.3)	0.6
PIRADS 4	-	-	-	46 (33.1)	77 (28.4)	0.4
PIRADS 5	-	-	-	73 (57.9)	88 (60.7)	0.7
GG≥3 TB per lesion, N (%)						
PIRADS 3	-	-	-	8 (8.6)	19 (5.8)	0.3
PIRADS 4	-	-	-	41 (29.5)	56 (20.7)	0.0502
PIRADS 5	-	-	-	67 (53.2)	70 (48.3)	0.5
Maximum cancer core length SB + TB (mm), median (IQR)	2 (1-6)	3 (1-6)	0.5	11 (7-13)	8 (5-12)	<0.001
Maximum cancer core length SB (mm), median (IQR)	2 (1-6)	3 (1-6)	0.5	7 (4-11)	6 (3-9)	0.008
Maximum cancer core length TB (mm), median (IQR)	-	-	-	11 (7-13)	8 (5-11)	<0.001
Maximum cancer core percent SB + TB (%), median (IQR)	10 (7-43)	20 (5-40)	0.9	80 (60-95)	60 (30-80)	<0.001
Maximum cancer core percent SB (%), median (IQR)	10 (7-43)	20 (5-40)	0.9	60 (30-80)	40 (20-70)	<0.001
Maximum cancer core percent TB (%), median (IQR)	-	-	-	80 (60-91)	60 (30-80)	<0.001

TRUS = transrectal ultrasound; PIRADS = Prostate Imaging Reporting and Data System; MRI = magnetic resonance imaging; No. = number; IQR, Interquartile Range; PCa = prostate cancer; CSPCa = Clinically significant PCa (Grade Group > 1); SB = systematic biopsy; TB = target biopsy.

similar (1.9% vs 1.7%, p=0.8) between the TP and TR groups, respectively (Supplementary Table-4, see more). Four patients in the TR group and one patient in the TP group developed sepsis. Clavien-Dindo Grade ≥III complications occurred in 2 (0.4%) and 3 (0.3%) of patients in the TP and TR groups (p=0.9), respectively.

DISCUSSION

In this single-center prospective database study, TP PBx detected more CSPCa and high-grade PCa than TR PBx in a large ethnically diverse and multiracial cohort in univariable analysis; however, the biopsy approach was not an independent predictor on multivariable analyses. The TP approach yielded higher cancer core length and percent involvement in TB and was an independent predictor on multivariable analyses. The median procedure time was lower for the TR group, while patients' self-reported pain levels and complications were similar between both approaches. Hence, while being similar in safety and tolerance, TP PBx is potentially histologically more informative than TR PBx.

Recent randomized trials have compared the TP and TR approaches. Mian et al. assessed the efficacy and complications between TP and TR in a single center setting in the US (8, 9). CSPCa detection rates (43.2% vs 47.1; odds ratio [OR], 1.17; 95% Confidence Interval [CI], 0.88-1.55) and complications (2.7% vs 2.6%, OR, 1.06; 95% CI, 0.43 to 2.65; p=.99) were similar for TP and TR, respectively. No patients in either group developed sepsis. Similarly, in Hu et al. PREVENT trial (7), CSCPa was detected in 53% and 50% of patients for TP and TR PBx,

Table 3 - Univariable and Multivariable logistic regression analyses for clinically significant cancer detection on transperineal versus transrectal MRI/TRUS fusion prostate biopsy.

	Univariate			Multivariate		
Variables	OR	CI (95%)	p	OR	CI (95%)	p
Age, year	1.06	1.04-1.07	<0.001	1.06	1.04-1.08	<0.001
Family History PCa	1.02	0.80-1.29	0.87			
Biopsy history			<0.001			<0.001
Previous Negative biopsy vs Naïve	0.48	0.37-0.62		0.58	0.41-0.80	
Previous Positive biopsy vs Naïve	1.03	0.79-1.34		1.17	0.84-1.63	
PSA, ng/mL	1.05	1.03-1.07	<0.001	1.08	1.05-1.11	<0.001
PSA density*, ng/mL²	1.07	1.06-1.08	<0.001			
Race			0.16			0.01
Asian vs NH-White	0.84	0.60-1.18		0.68	0.44-1.05	
Hispanic vs NH-White	0.86	0.64-1.17		0.91	0.63-1.35	
Black vs NH-White	1.60	0.99-2.63		2.49	1.31-4.74	
Others vs NH-White	0.90	0.66-1.23		0.81	0.55-1.19	
DRE, suspicious vs non-suspicious	4.1	3.20-5.29	<0.001	3.18	2.3-4.4	<0.001
Prostate Volume, cc	0.983	0.980-0.987	<0.001	0.979	0.974-0.983	<0.001
No. MRI lesions	1.92	1.69-2.19	<0.001			
MRI lesion size, mm	1.06	1.04-1.08	<0.001			
PIRADS 3-5 vs PIRADS 1-2	8.63	6.33-12.01	<0.001	4.74	2.99-7.60	<0.001
MRI lesion location, n (%)						
Non-anterior vs anterior	0.43	0.34-0.55	<0.001	0.90	0.66-1.23	0.5
MRI lesion location, n (%)						
Non-apical vs apex	0.72	0.55-0.93	0.01	1.29	0.92-1.80	0.13
No. TB cores taken	1.35	1.29-1.41	<0.001	1.15	1.08-1.24	<0.001
Prostate biopsy approach TP vs TR	1.25	1.01-1.56	0.04	1.04	0.78-1.39	0.78

PIRADS = Prostate Imaging Reporting and Data System; MRI = magnetic resonance imaging; OR = odds ratio; CI, confidence interval; PCa = prostate cancer; CSPCa = Clinically significant PCa (Grade Group ≥ 2); DRE = digital rectal examination; DRE = digital rectal examination; NH = non-Hispanic.
*PSA density was calculated per 0.01 unit.

respectively (adjusted difference 2.0%; 95% CI −6.0%, 10%). No significant difference in infectious complications was noted between approaches (difference, −1.4%; Newcombe hybrid score 95% CI −0.3, 3.2; p = 0.059), although no cases of infection occurred in the TP arm. Ploussard et al. trial (10) reported no statistically significant difference in CSPCa detection rates for MRI-targeted TP or TR PBx (47.2% vs 54.2%, p=0.62).

However, a major limitation is the patient selection in these trials, which consists overwhelmingly of white males. Homogeneous patient populations limit the external validity of the results, thereby potentially leading to inadequate management of PCa (27, 28). For instance, Black men tend to present with more aggressive prostate cancer at initial diagnosis, are less likely to receive definitive treatment, and have a higher incidence and mortality rate from prostate cancer compared to other races (29). Hence the importance of accurate early detection in this population. In contrast, it has been suggested that Asian men are less likely to be diagnosed with prostate cancer compared to white men, independent of PSA levels, suggesting that biological, genetic, or environmental factors may influence the disease's development (30). Therefore, a strength of this study is having assessed the PBx approach comparison in a diverse cohort including Asians, Black, Latino, White and Other patients.

The higher detection rates of CSPCa and high-grade PCa in the univariable analysis for the TP group can be attributed to a higher number of lesions on MRI and greater lesion sizes among these patients. To address this imbalance, multivariable logistic regression analyses were conducted. Interestingly, TP patients had more anterior lesions compared to TR patients, despite biopsy approach selection not being based on lesion location. In the trial by Ploussard et al. (10), MRI-targeted TR significantly detected more posterior CSPCa than the TP approach (59.0% vs. 44.3%, p=0.04), while TP detected more CSPCa in anterior lesions, although this was not statistically significant (40.6% vs. 26.5%, p=0.22). Despite these findings, the sample size might have been insufficient to detect differences in subgroup detection rates, such as lesion location; thus, further studies with larger cohorts are needed to elucidate these results. Additionally, even though the study was randomized, there were more PIRADS 5 cases in the TR group than in the TP group, which could have influenced the outcomes. A meta-analysis involving 8,826 patients demonstrated a higher detection rate of CSPCa in the anterior region with TP PBx, both in a per-lesion analysis (p=0.03) and a per-patient analysis (p<0.001)(5). In the present study's cohort, there was no statistically significant difference between TP and TR PBx CSPCa detection rates in anterior lesions.

In this study, TP PBx was histologically more informative than the TR PBx in both univariable and multivariable analysis. Greater cancer core length and percentage were evidenced in TP target biopsy. A multicenter study of 1293 patients that underwent MRI-targeted and systematic TR or TP PBx reported that downgrading at radical prostatectomy was associated with a TR approach, lower cancer core length and percent on systematic PBx (all p≤0.03) (31). On multivariable analysis, higher maximum cancer core length and TP PBx were associated with a lower rate of downgrading. This suggests that TP PBx could play a role in reducing overtreatment in PCa management, underscoring the importance of its greater histological informativeness. Additionally, as more experience with focal therapy for PCa develops, the importance of histologically detailed biopsies remains to be determined and may have implications for patient selection or eligibility for focal therapy in the future.

Although randomized trials have shown that rectal cleansing with iodine may decrease TR PBx infectious complications (32), more recent studies comparing TP versus TR PBx have not adopted this practice (7, 8). Similarly, we opted not to perform povidone-iodine preparation before TR or TP PBx. Since complications, procedural time, and patient-experienced pain levels under local anesthesia are similar for both approaches and CSPCa detection rates are comparable, the choice between TR and TP PBx can be based on patient preference. However, TP PBx has the advantages of avoiding rectal perforation, not requiring antibiotic prophylaxis in most patients, and potentially being more histologically informative with superiority for anterior lesions (27). The next challenge is to widely implement and increase

training in the TP approach across academic and community centers worldwide, ultimately providing a more patient-centered approach for prostate biopsies.

This study has limitations. This is a single-center experience, therefore limiting the generalizability of the results. Nonetheless, the diverse patient population and the large cohort are a strength of this study. Although the data was collected on a prospectively maintained PBx database, this is a retrospective and non-randomized study. Nevertheless, possible selection bias or confounders were addressed by using multivariable logistic and linear regression models. Overall, the present study's results resonate with the conclusions reported in the clinical trials (7, 9, 10). However, this study evidenced that Black Race is an independent predictor for CSPCa detection, irrespective of the PBx approach, which wasn't demonstrated in prior trials. Additionally, the data support that TP PBx is histologically more informative than the TR PBx.

CONCLUSIONS

In a large, diverse, multiracial and ethnic cohort, Black race was an independent predictor for CSPCa detection, but neither the biopsy approach nor anterior lesions on MRI were independent predictors. TP and TR PBx yielded similar CSPCa and high-grade PCa detection rates. TP PBx was histologically more informative, providing greater cancer core length and percent involvement on target biopsy samples.

ABBREVIATIONS

ADC = apparent diffusion coefficient
CSPCa = Clinically significant cancer
DCE = dynamic contrast-enhanced
DRE = digital rectal examination
DWI = diffusion-weighted imaging
GG = Grade Group
ISUP = International Society of Urological Pathology
mpMRI = Multiparametric MRI
MRI = magnetic resonance imaging
PBx = prostate biopsy
PCa = prostate cancer
PIRADS = Prostate Imaging-Reporting and Data System
PSA = Prostate specific antigen
PSAD = Prostate specific antigen density
PV = Prostate volume
SB = Systematic biopsy
T2W = T2-weighted
TB = Target biopsy
TP = Transperineal
TR = Transrectal
TRUS = Transrectal ultrasound

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CONFLICT OF INTEREST

Inderbir Gill has equity interest in OneLine Health and Karkinos
Andre Luis Abreu is a consultant for Koelis, a speaker for EDAP, and a proctor for Sonablate
Other authors do not have any competing interests

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Correspondence address:
Andre Luis Abreu, MD
Director of Image-Guided Surgery, Focal Therapy and
Artificial Intelligence for Prostate Cancer
USC Institute of Urology
E-mail: andre.abreu@med.usc.edu

EDITORIAL

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MRI Changed the diagnosis of prostate cancer

Luciano A. Favorito [1, 2]

[1] Unidade de Pesquisa Urogenital - Universidade do Estado do Rio de Janeiro - Uerj, Rio de Janeiro, RJ, Brasil; [2] Serviço de Urologia, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brasil

COMMENT

In cases of prostate cancer investigation MRI can be used during a prostate biopsy to help guide the needles into the prostate. This paper of the group of Dr. Abreu and Gill (1) shows the importance of MRI in the prostate cancer diagnosis. In the last years several papers studied the prostate biopsy showing the superiority of the perineal MRI/TRUS Fusion-Guided Prostate Biopsy (2-4). The group of the University of Southern California compared transperineal (TP) vs transrectal (TR) magnetic resonance imaging (MRI) and transrectal ultrasound (TRUS) fusion-guided prostate biopsy (PBx) in a large, ethnically diverse and multiracial cohort with 1491 patients, with 480 undergoing TP and 1011 TR PBx and concluded that in a large and diverse cohort, black race, but not the biopsy approach, was an independent predictor for clinical significant prostate cancer (CSPCa) detection. TP and TR PBx yielded similar CSPCa detection rates, however the TP PBx was histologically more informative.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Luciano Alves Favorito, MD, PhD
Unidade de Pesquisa Urogenital, Departamento de Anatomia da Univer. do Est. do Rio de Janeiro – UERJ
Rua Professor Gabizo, 104/201
Rio de Janeiro, RJ, 20271-320, Brasil
Telephone: +55 21 2264-4679
E-mail: lufavorito@yahoo.com.br

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Luciano Alves Favorito
https://orcid.org/0000-0003-1562-6068

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A multi-faceted exploration of unmet needs in the continuing improvement and development of fertility care amidst a pandemic

Monica Augustyniak [1], Giovanni Coticchio [2], Sandro C. Esteves [3,4,5], Markus S. Kupka [6], Chen Hong [7], Anita Fincham [8], Patrice Lazure [1], Sophie Péloquin [1]

[1] AXDEV Group Inc., Brossard, QC, Canada; [2] IVIRMA Global Research Alliance, 9.baby, Bologna, Italy; [3] ANDROFERT, Clínica de Andrologia e Reprodução Humana, Campinas, SP, Brasil; [4] Departamento de Cirurgia, Divisão de Urologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil; [5] Faculty of Health, Aarhus University, Aarhus, Denmark; [6] Ludwig-Maximilians -University, Munich, Germany; [7] Shanghai First Maternity and Infant Hospital, Shanghai, China; [8] Fertility Europe, Brussels, Belgium

ABSTRACT

Purpose: The continuous improvement and development of fertility care, internationally, requires ongoing monitoring of current delivery processes and outcomes in clinical practice. This descriptive and exploratory mixed-methods study was conducted in eight countries (Brazil, China, France, Germany, Italy, Mexico, Spain and the United Kingdom) to assess the unmet needs of fertility patients (male and female), and existing challenges, barriers and educational gaps of physicians and laboratory specialists involved in human fertility care during the COVID-19 pandemic.

Materials and Methods: The study was deployed sequentially in two phases: 1) in-depth 45-minute semi-structured interviews (n=76), transcribed, coded and thematically analysed using an inductive reasoning approach, 2) an online survey (n=303) informed by the findings of the qualitative interviews, face validated by experts in reproductive medicine, and analysed using descriptive and inferential statistical methods.

Results: The integrated results of both phases indicated numerous areas of challenges, including: 1) investigating male-related infertility; 2) deciding appropriate treatment for men and selective use of assisted reproductive technology; and 3) maintaining access to high-quality fertility care during a pandemic.

Conclusions: The paper presents a reflective piece on knowledge and skills that warrant ongoing monitoring and improvement amongst reproductive medicine healthcare professionals amidst future pandemics and unanticipated health system disruptions. Moreover, these findings suggest that there is an additional need to better understand the required changes in policies and organizational processes that would facilitate access to andrology services for male infertility and specialized care, as needed.

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Monica Augustyniak
https://orcid.org/0000-0003-2825-5497

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INTRODUCTION

It may be worthwhile to revisit a recent event that substantially disrupted health systems and reproductive medicine at a global scale: the COVID-19 pandemic. The first seven cases of hospitalized pneumonia due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, also known as COVID-19) were reported and investigated in Wuhan Jin Yin-Tan Hospital, China, in December 2019 (1). Shortly after, in March 2020, the World Health Organization (WHO) announced a state of pandemic (2). Outbreak measures were recommended by professional health societies and installed by national public health authorities including suspension of healthcare services considered "non-urgent". Fertility care fell under this categorization.

Professional associations in reproductive medicine recommended discontinuation of fertility care services, particularly the use of assisted human reproductive technology (ART) and andrological evaluation, for new patients and those without cancer diagnoses (3, 4). This recommendation was informed by the lack of evidence pertaining to the effect of COVID-19 infection on pregnancy and neonatal outcomes, and the need to ensure physical distancing as preventative and precautionary measures. The impact of this recommendation was quickly felt.

In 2020, a survey administered to 207 individual fertility care centres across 97 countries showed that 83% of the respondents reported no or limited access to ART treatments in their country, and 40% reported changes in policies regarding fertility treatments offered to their patient population (5). Patients' psychological distress associated with a drastic lack of services, especially for those with a narrow window for successful conception via ART, was reported (6-8).

A call to reconsider and adapt policies surrounding access to ART was made to ensure reproductive care was not "unfairly curtailed" to low-prognosis patients (9-13). These urgent requirements for change were only additions to previously identified challenges in the field: ensuring evaluation of male-related factors prior to selecting an ART procedure, such as intracytoplasmic sperm injection (ICSI) (14, 15); reaching consensus on the appropriate stage of embryo cryopreservation or vitrification (16); using ART in alignment with best-practice guidelines (17); providing effective psychological support for patients (18); and tailoring communication to meet patient needs (17).

Amidst all these changes and growing needs, healthcare professionals (HCPs) involved in reproductive medicine are also expected to stay abreast of, and integrate continuing advances in their practice as needed, including: integrating updated recommendations and guidelines (10, 19-22), emerging assessments tools (23, 24), and newly available treatments, such as those addressing aetiologies of male infertility (25).

With the aim of supporting reproductive medicine HCPs in effectively meeting their professional expectations during and beyond the COVID-19 pandemic, via evidence-based continuing medical education (CME), professional development (CPD) activities and other types of health system interventions, this study assessed priority needs for improvement in fertility care from the perspective of patients (male and female), and remaining challenges, barriers and educational gaps affecting physicians and laboratory specialists involved in ART and the treatment of male and/or heterosexual couples' infertility.

MATERIALS AND METHODS

An exploratory sequential mixed-methods study was conducted, involving a triangulation of data sources, methods, and interpretation viewpoints (26, 27). This approach was selected to obtain a fulsome capture of the examined phenomena, in line with the study objectives, superior to what could be obtained with quantitative or qualitative approaches alone (28). An equal priority was given to both methods. The first phase (June 2021-August 2021) involved qualitative interviews with HCPs (physicians and laboratory specialists involved in ART and male infertility) and patients (male and female), to explore the context and meaning of challenges experienced in fertility care. The second phase (November 2021) used a quantitative survey informed by the findings of the qualitative interviews to measure the frequency and magnitude of gaps and challenges,

in a distinct and larger sample of HCPs. The final phase involved integrating all findings to identify converging themes (26, 29), and underlying educational gaps. Gaps were defined as the discrepancy between current and ideal states of knowledge, skills, beliefs, and performance of HCPs (30). The study protocol was reviewed and approved by Veritas Independent Review Board Inc. in accordance with ethical guidelines and regulations of the countries in which the study was conducted.

Recruitment

E-mail invitations describing the study were sent to prospective participants in Brazil, China, France, Germany, Italy, Mexico, Spain, and the United Kingdom (UK). For each study phase, unique panels of HCPs and patients registered to receive invitations for healthcare research were used. Panels operated in compliance with the International Chamber of Commerce (ICC) and European Society for Opinion and Marketing Research (ESOMAR) guidelines. Invitations included a link to an online screening questionnaire and consent form. Phase 1 inclusion criteria were: 1) male or female patients, 18 to 55 years of age, actively seeking fertility care, and having attempted conception with ART at least once; or 2) actively practicing physicians specialised in reproductive medicine, reproductive endocrinology, obstetrics and/or gynecology (OBGYN) or andrology, with a minimum of five years in practice, involved in the diagnosis, treatment and/or management of infertility, and at least 500 ART-related procedures conducted over the last 12 months; or 3) actively practicing embryologists, andrology laboratory specialists, biologists, or microbiologists with a minimum of five years in practice, involved in the manipulation of human gametes or embryos of at least 10 patients per year for the purpose of ART. Phase 2 was conducted with HCPs only. Inclusion criteria were similar to phase 1, except: physicians could specialise in reproductive urology, all were required to provide care to male patients; a minimum of three years in practice experience and yearly caseload of at least 100 patients undergoing ART. All participants provided informed consent prior to enrolling. Purposive sampling was applied on an ongoing basis to ensure a variety of perspectives and profiles were obtained across samples (31).

Phase 1: Qualitative Interviews (March – September 2021)

An interview guide was developed based on challenge areas identified in the literature (Appendix-1). Questions were open-ended to elicit robust, descriptive responses, and allow for discussion of experiences and perspectives relevant to different professions and patients (32). Probes were used when explanations or contextualisation were needed. Final materials were translated into French, German, Italian, Mandarin, and Portuguese.

Semi-structured 45-minute interviews were conducted with trained moderators over a secure conference call in the participant's language, and recorded upon consent. Recordings were transcribed and imported into NVivo Version 12 software (QSR International Pty Ltd., 2021) for coding or organisation into a framework of relevant topics. If unanticipated but relevant content emerged from data analysis, a new code was created to integrate the topic into the analysis framework. Three researchers, including co-author MA coded the transcripts. Inter-coder reliability test results demonstrated fair consistency and reliability (Agreement rate > 90% amongst coders) (33). Data were thematically analysed using an inductive reasoning approach (34).

Phase 2: Quantitative Survey (October – December 2021)

A 20-minute survey was developed by co-authors MA, PL and SP based on phase 1 findings. The HCP survey was face-validated by subject matter experts in reproductive medicine (co-authors GC, SE, MK, CH) and a patient organisation representative (co-author AF). The survey consisted of twenty-two closed-ended questions in the form of rating with five-point Likert-type scales (e.g., 1-no knowledge/skill, 2-basic, 3-intermediate, 4-advanced, 5-expert knowledge/skill) or multiple-choice response options, summing up to 174 survey items. Survey items were split between physicians caring exclusively for males; physicians caring for both sexes; and laboratory specialists and assessed knowledge, skill, beliefs (or attitudes), and performance in clinical practice (30, 35). A clinical case question was included to help evaluate HCP decision-making when investigating and treating a couple's infertility.

The minimal targeted sample size (n=176) for the survey was calculated to reach a statistical power of 0.8 with α=0.05 and a large effect size (Cohen's w=0.5) for a 2x4 chi-square test (36), to account for comparison between four regions: South America, Western Europe, Southern Europe and Asia (n=44 per region). With the aim of strengthening descriptive comparisons by country, the final sample size was permitted to increase until survey closure. Survey responses were imported into SPSS Statistics (Version 27.0, IBM Corp., Armonk, NY, USA) for frequency and crosstabulation analysis with chi-square statistical tests. Appendix-2 presents full questions and responses discussed in the manuscript, as well as how each survey response was transformed for crosstabulation analysis.

Data Integration and Trustworthiness

Integration of mixed methods was achieved by ensuring phase 1 findings informed the development of measures for phase 2 (27). In the reporting of findings, quotes representative of the identified challenge were further integrated by the co-authors to articulate meaning and context, while statistics from phase 2 were integrated to quantify the extent to which gaps and barriers were identified. The integration of mixed methods was first completed by researchers MA, PL and SP, and then reviewed in collaboration with co-authors CG, SCE, MK, CH and AF via online discussions.

RESULTS

Seventy-six interviews and 303 surveys were completed (Table-1). Phase 1 included female (n=23) and male (n=5) patients; the majority of who were over the age of 34 (F:70%; M:100%) and had received assisted conception, such as *in vitro* fertilization (IVF) (F:91%, M:80%) and medication, such as gonadotropins (F: 91%, M:60%). Phase 1 physicians were specialised in OBGYN (42%, 10/24), reproductive medicine (38%, 9/24) or endocrinology (21%, 5/24), while laboratory personnel included embryologists (70%,16/23), biologists (17%, 4/23), laboratory managers (9%, 2/23) and microbiologists (4%, 1/23). Phase 2 physicians were specialised in reproductive medicine (55%, 124/224), endocrinology (24%, 53/224), OBGYN (29%, 66/224), reproductive urology (17%, 39/224) and andrology (15%, 15/224), while laboratory personnel included embryologists (52%, 51/99), laboratory specialists (43%, 43/99) and biologists (5%, 5/99). The median number of ART-related procedures conducted by HCPs was 550 per year in phase 1, and 300 per year in phase 2.

The following themes and areas of challenges for HCPs emerged from the integration of results from both phases: 1) investigating male-related infertility; 2) deciding appropriate treatment for men with selective use of ART-related procedure; and 3) maintaining access to high-quality fertility care during a pandemic. Figure-1 outlines each area, underlying gaps and barriers hindering optimal care.

1) Challenges Investigating Male-Related Infertility

A knowledge gap of the extent of male contribution to couples' infertility was found, in addition to skill gaps and preconceived notions deterring a thorough investigation of male infertility. Over a third (38%, 123/323) of surveyed HCPs responded that males contributed to 30% or less of all cases of couples' infertility. A greater proportion of HCPs from China (58%, 32/55), Mexico (69%, 27/39) and Germany (43%, 16/37) demonstrated this knowledge gap, compared to HCPs from other countries (Table-2). On average, 31% (100/318) of physicians rated their skill level as less than advanced or expert (i.e., as none, basic or intermediate) when investigating male causes of infertility. The percentage of participants reporting suboptimal skill levels was greatest amongst physicians specialised in reproductive medicine, endocrinology or OBGYN (38%, 65/169) than reproductive urology or andrology (11%, 6/53). Patient interviews indicated HCPs lack consideration of the factors, other than female age, that might contribute to difficulty conceiving:

> *It was my wife who wanted to get pregnant. Thus, I let her organise everything. All was focused on her. All the tests that were done were focused on her. When we visited the last doctor, he focused on me instead. He explained that it was me who was unable to have children.*
> – Male patient (45 years of age), Mexico

Table 1 - Sample Demographics.

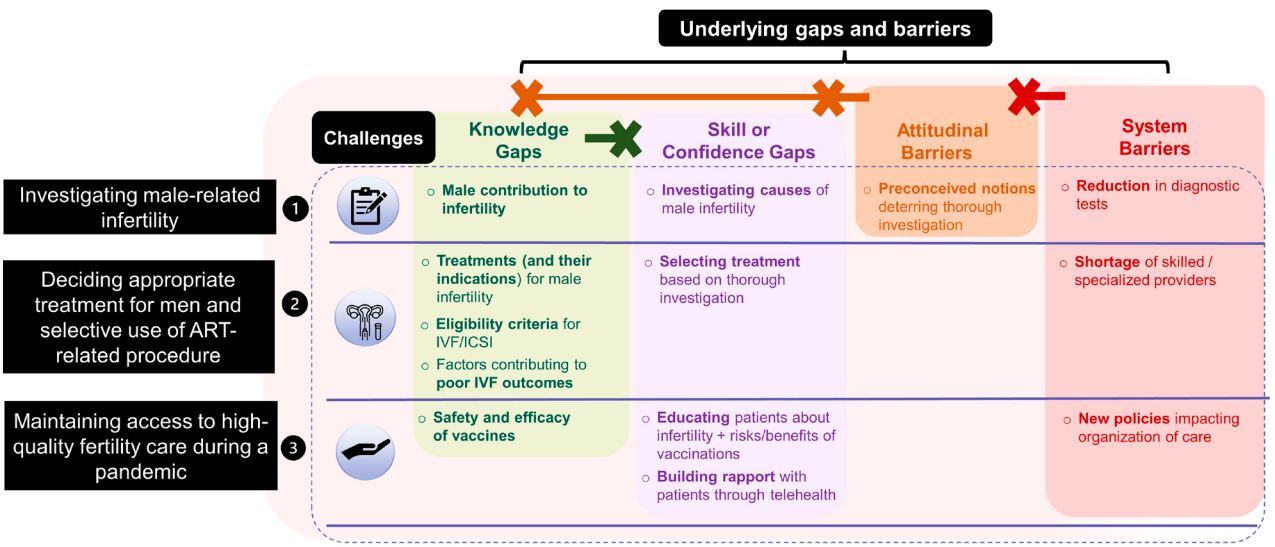
Demographics		Phase 1 (Qualitative Interviews) n=76			Phase 2 (Quantitative Survey) n=323	
		PHYS [a] (n=24)	LAB [b] (n=24)	PX [c] (n=28)	PHYS [a] (n=224)	LAB [b] (n=99)
Region	**South America**	6 (26%)	6 (26%)	8 (29%)	54 (24%)	24 (24%)
	Western Europe	9 (38%)	9 (38%)	9 (32%)	75 (34%)	36 (36%)
	Southern Europe	6 (25%)	6 (25%)	8 (29%)	54 (24%)	24 (24%)
	Asia	3 (13%)	3 (13%)	3 (11%)	40 (18%)	15 (15%)
Country	**Brazil**	3 (13%)	3 (13%)	4 (14%)	27 (12%)	12 (12%)
	China	3 (13%)	3 (13%)	3 (11%)	40 (18%)	15 (12%)
	France	3 (13%)	3 (13%)	3 (11%)	26 (12%)	12 (12%)
	Germany	3 (13%)	3 (13%)	3 (11%)	25 (11%)	12 (12%)
	Italy	3 (13%)	3 (13%)	4 (14%)	27 (12%)	12 (12%)
	Mexico	3 (13%)	3 (13%)	4 (14%)	27 (12%)	12 (12%)
	Spain	3 (13%)	3 (13%)	4 (14%)	27 (12%)	12 (12%)
	UK	3 (13%)	3 (13%)	3 (11%)	25 (11%)	12 (12%)
Setting	**Community standalone****	7 (29%)	9 (38%)	-	91 (41%)	55 (56%)
	Community hospital	7 (29%)	8 (33%)	-	54 (24%)	37 (37%)
	Academic hospital	10 (42%)	7 (29%)	-	79 (35%)	7 (7%)
Years of practice	**3-10 years***	5 (21%)	8 (33%)	-	81 (36%)	51 (52%)
	11-20 years	14 (58%)	12 (50%)	-	110 (49%)	42 (42%)
	> 20 years	5 (21%)	4 (17%)	-	33 (15%)	6 (6%)
Age	**25-34 years**	-	-	7 (25%)	-	-
	35-44 years	-	-	16 (57%)	-	-
	> 44 years	-	-	5 (18%)	-	-
Sex	**Female**	15 (63%)	11 (46%)	23 (82%)	97 (43%)	60 (61%)
	Male	9 (38%)	13 (54%)	5 (18%)	124 (55%)	37 (37%)

[a] PHYS: Reproductive medicine specialists, endocrinologists, obstetricians and gynecologists, reproductive urologists or andrologists involved in ART; [b] LAB: Microbiologists, biologists or embryologists involved in the manipulation of gametes or embryos for the purpose of ART; [c] PX: Male and female patients of fertility age, with full reproductive organs, seeking care due to difficulty conceiving and have attempted at least once ART.

* Minimum years of practice for phase 1 was 5 years, compared to 3 years for phase 2.

** Standalone community practice settings included: single-specialty or solo practice in andrology, gynecology or fertility care, as well as multi-speciality clinics or centres outside of hospitals.

Figure 1 - Three challenge areas in fertility care with underlying gaps and barriers.



Description: Three challenge areas were identified by this study with related knowledge and skill gaps, attitudinal barriers (e.g., beliefs that hindered optimal practice), and system-level barriers. Knowledge gaps can hinder the appropriate acquisition and demonstration of skills; attitudinal barriers can hinder the acquisition and development of both knowledge and skills, while system-level barriers can influence all individual-level factors (i.e., knowledge, skills, and attitudes).

Of physicians involved in the care of both sexes, 32% (60/188) reported not always considering the health of male partners when investigating and treating infertility. An average of 43% (138/323) of surveyed HCPs agreed or strongly agreed with the statement "causes of male infertility are simpler to investigate than female infertility." The agreement was less prominent among physicians and laboratory specialists in Italy and Spain (29%, 23/78, Appendix-3).

2) Challenges in Deciding Appropriate Treatment for Men and Selective Use of ART-related procedures

Knowledge gaps regarding available treatments to address male infertility, and uncertainty about ART eligibility criteria were found. Procedures and treatments where the highest proportion of physicians rated their knowledge as less than advanced or expert were: microsurgical reconstruction of the male genital tract (61%, 133/218), transurethral resection of ejaculatory ducts (58%, 126/217), techniques for varicocele repair (48%, 105/219), and empirical medical treatment with selective estrogen receptor modulators, antioxidants and gonadotropin for males (47%, 102/219). When considering multiple potential treatments for male patients, 43% (70/162) of specialists in reproductive medicine, endocrinology, and OBGYN had a knowledge gap, compared to 10% (5/50) in reproductive urology and andrology (p<0.001).

A majority (85%, 275/323) of surveyed HCPs across all countries either were unsure of, or incorrectly agreed with the statement that "even very low rates of sperm morphology have poor predictive power" for IVF, and "poor embryonic development in an IVF cycle is an indication for ICSI", despite evidence demonstrating the contrary (Appendix-3). Interviewed HCPs expressed challenges justifying a recommendation for ICSI to patients when semen analysis results are close to the threshold for inhibition of semen function:

In 2010, WHO determined new standard parameters, e.g., 15 million sperms per mL, the mobility should be 32%, and at least 4% should be perfectly shaped. [...] *the threshold is so low that you say that everybody who gets below that, even in just one parameter, has a severe inhibition, thus an ICSI indication* [...] *It's really difficult to understand. It's difficult to explain this to the couple.*

– Physician (Reproductive Medicine), Germany

Table 2 – Percent of physicians with gaps in knowledge (K) and skill (S) by country.

Gap	Area of care	Countries (%, n)								
		South America		Western Europe			Southern Europe		Asia	Total Mean
		BR	MX	DE	FR	UK	IT	SP	CH	
K	Male contribution to infertility*	18% (7/39)	69% (27/39)	43% (16/37)	26% (10/38)	29% (11/37)	26% (10/39)	26% (10/39)	58% (32/55)	38% (123/323)
K	Eligibility for IVF and ICSI*	11% (3/27)	15% (4/27)	20% (5/25)	8% (2/26)	8% (2/24)	4% (1/26)	11% (2/27)	38% (15/39)	16% (35/221)
K	Microsurgical reconstruction of the male genital tract*	76% (19/25)	69% (18/26)	40% (10/25)	38% (10/26)	63% (15/24)	68% (18/27)	68% (18/27)	66% (25/38)	61% (133/218)
K	Transurethral resection of ejaculatory ducts*	88% (21/24)	70% (19/27)	24% (6/25)	44% (11/25)	63% (15/24)	52% (14/27)	68% (18/27)	58% (22/38)	58% (126/217)
K	Techniques for varicocele repair*	72% (18/25)	59% (16/27)	24% (6/25)	23% (6/26)	50% (12/24)	48% (13/27)	48% (13/27)	55% (21/38)	48% (105/219)
K	Empirical medical treatment with selective estrogen receptor modulators, antioxidants, and gonadotropin in males	65% (17/26)	33% (9/27)	47% (18/38)	48% (12/25)	54% (13/24)	41% (11/27)	56% (15/27)	47% (18/38)	47% (102/219)
K	Gonadotropin therapy for hormonal disorders in males*	74% (20/27)	26% (7/27)	33% (13/39)	23% (6/26)	26% (6/23)	41% (11/27)	44% (12/27)	33% (13/39)	36% (80/220)
K	Treatments for male patients that can improve IVF and ICSI outcomes*	31% (8/26)	30% (8/27)	16% (4/25)	27% (7/26)	25% (6/24)	19% (5/27)	33% (9/25)	50% (19/38)	30% (66/220)
K	Effectiveness of vaccines in preventing SARS-CoV-2 infections and limiting symptom severity	44% (15/34)	72% (28/39)	40% (14/35)	26% (10/38)	57% (21/37)	47% (17/36)	42% (15/36)	56% (29/52)	49% (149/307)
S	Investigating hypothalamic-pituitary axis dysfunction in males*	44% (12/27)	30% (8/27)	12% (3/25)	23% (6/26)	28% (7/25)	25% (6/24)	38% (10/26)	56% (22/39)	34% (74/219)
S	Investigating spermatogenic defects	42% (16/38)	18% (7/38)	24% (9/37)	34% (13/38)	36% 13/36)	32% (12/38)	44% (17/39)	48% (26/54)	36% (113/318)
S	Investigating ductal obstruction or dysfunction in males	44% (12/27)	44% (12/27)	24% (6/25)	32% (8/25)	44% (11/25)	32% (8/25)	44% (12/27)	41% (16/39)	39% (85/220)
S	Investigating infectious disease causes of infertility in males	33% (9/27)	19% (5/27)	16% (4/25)	23% (6/26)	52% (13/25)	35% (9/26)	52% (14/27)	44% (17/39)	35% (77/222)
S	Investigating systemic causes of infertility in males	37% (10/27)	18% (5/27)	20% (5/25)	19% (5/26)	17% (4/24)	19% (5/26)	41% (11/27)	41% (16/39)	28% (61/221)
S	Counselling patients on the safety and efficacy of available vaccines (including ones for SARS-CoV-2) *	11% (3/27)	40% (10/25)	28% (7/25)	27% (7/26)	42% (10/24)	42% (10/24)	44% (12/27)	67% (26/39)	39% (85/217)
S	Discussing with patients' evidence regarding the risks versus benefits of SARS-CoV-2 vaccines on pregnancy and birth outcomes *	15% (4/27)	41% (11/27)	20% (5/25)	31% (8/26)	40% (10/25)	48% (12/25)	41% (11/27)	67% (26/39)	40% (89/221)
S	Building rapport with patients via telehealth during an initial evaluation *	8% (2/25)	56% (14/25)	16% (4/25)	32% (8/25)	12% (3/25)	44% (11/25)	22% (4/27)	48% (19/40)	31% (67/217)

K (Knowledge gap); S (Skill gap); BR (Brazil); MX (Mexico); DE (Germany); FR (France); UK (United Kingdom); IT (Italy); SP (Spain); CH (China). *Asymptotic significance (2-sided) < 0.05 for 2x4 (gap X region) crosstabulation with Pearson Chi-Square statistical test.

Uncertainty about the eligibility criteria for an ART-related procedure was further confirmed by survey responses showing that 35% (113/323) of HCPs selected "ICSI" as opposed to "full andrological evaluation by a specialist" as the next best course of action for a case of a 45-year-old male and a 32-year-old woman who had had a previous IVF cycle resulting in 20 mature oocytes but poor embryonic development (Appendix-4).

3) Challenges Maintaining Access to High-Quality-Fertility Care During a Pandemic

Enduring system-level changes resulting from the COVID-19 pandemic were reported by participants. Surveyed HCPs reported staffing shortages (47%, 153/323), limited access to laboratories for specialised diagnostic testing (32%, 102/323), cancellation of certain diagnostic tests (e.g., fallopian tube examination) from standard clinic procedures (26%, 84/323), and the introduction of changes to patient triaging policies (38%, 85/224). These challenges are illustrated by country in Figure-2. Interviewed HCPs indicated that these institutional and organizational-related changes limited capacity to investigate and care for couples' infertility:

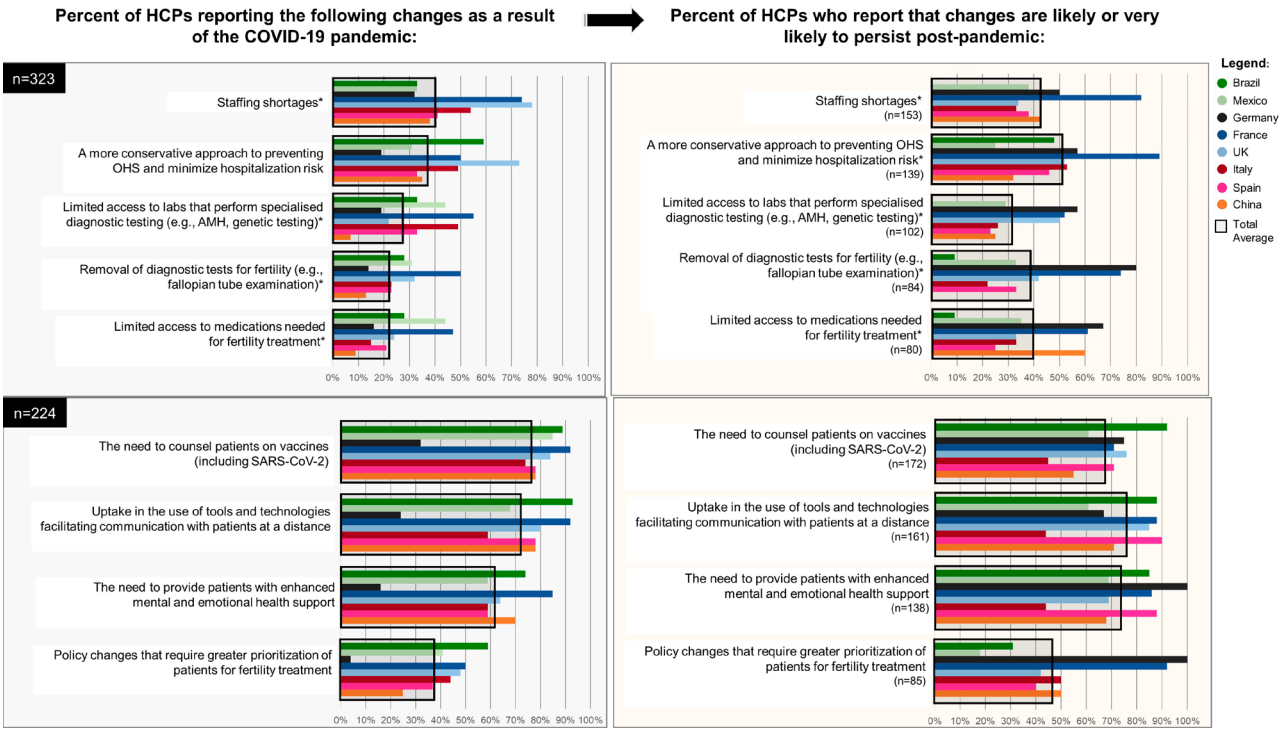
> *At a certain point, we had no time, because of all the extra bureaucratic work we had to do,* [including] *COVID procedures. In the past, we would execute tests to evaluate tubal functions,* [but] *at a certain point, we had to eliminate such testing. I think we haven't reintroduced them yet.*
> – Physician (OBGYN), Italy
>
> *The real impact was the prioritisation of patients. This is what had an impact on our activity. We were forced to adopt this strategy where we had to rank the degree of urgency of patients... When it comes to the protocol itself, it did not change. It is only the ranking that changed and giving priority to certain patients is something that is really difficult to do.*
> – Laboratory Specialist, France

Additional expected professional competencies that emerged during the COVID-19 pandemic and expected to remain post-pandemic (Figure-2), especially amongst physicians, included: the ability to counsel patients regarding the impact of SARS-CoV-2 vs. vaccines on fertility (77%, 172/224), increased use of telemedicine (72%, 161/224), enhanced provision of mental health and emotional support (62%, 138/224). Over 40% of surveyed HCPs (n=307) in all countries, except for France (31%, 8/26), rated their knowledge of the effectiveness of vaccines available to prevent SARS-CoV-2 infections and limit symptom severity at suboptimal levels. Over 40% of physicians in China, Spain, Italy, Mexico, and the UK reported suboptimal skill levels when discussing evidence of the risks versus benefits of SARS-CoV-2 vaccines on pregnancy and birth outcomes with patients (Table-2). Despite using telemedicine and recognising the need for patient support, participants in China (48%, 19/40), France (32%, 8/25), Italy (44%, 11/25), and Mexico (56%, 14/25) reported suboptimal skill levels when building rapport with patients via telehealth during initial evaluations.

DISCUSSION

While the COVID-19 pandemic has officially ceased in May 2023, the repercussions of this event are likely to persist and warrant a call for timely and tailored support for HCPs aiming to meet the continued and complex needs of patients moving along their reproductive journey. This study identified three challenge areas with underlying educational gaps (i.e., gaps in knowledge and skills) and barriers (i.e., beliefs or institutional/organizational level factors acting as hindrances) that could strategically inform tailored CME and CPD activities supporting physicians and laboratory specialists involved in ART and male infertility, in relation to: 1) investigating male-related infertility; 2) deciding appropriate treatment for men and selective use of ART-related procedure; and 3) maintaining high-quality fertility care during a pandemic. Identified challenges were reported by a substantial proportion of HCPs (>30%), both surveyed and interviewed, and corroborated with the interviews of patients who sought fertility care before and during the pandemic. These findings also align with challenges identified in pre-pandemic research, which strengthens the relevancy of the findings identified by this study (5-8, 14-18). Commonalities found across per-

Figure 2 - Percent of HCPs reporting changes as a result of the COVID-19 pandemic, and those likely to persist post-pandemic.



spectives, phases, and published literature, support the argument that the challenges, barriers and educational gaps identified in this study are likely present in the broader population of fertility professionals and merit immediate action (28, 29).

An important topic investigated in this study, beyond the impact of COVID-19 on access to fertility care, is the ongoing dismissal of male-related factors of infertility, that can lead to inappropriate selection and use of an ART-related procedure, such as ICSI (14, 37-39). This concern has been previously raised through an analysis of registry data from 79 countries, which indicated a steady increase in the number of ART-related procedures delivered globally over the last two decades, most of which have been ICSI-induced (40). This study provides evidence that physicians may recommend ICSI before rigorously investigating male's potential contribution to a couple's infertility. The following risk factors and indicators of male infertility can be missed by physicians: visual disturbances, lubricant use during sexual intercourse, and bariatric surgery (41). This study indicates a need to enhance HCPs' knowledge of male contribution to couples' infertility, including possible aetiologies of male infertility requiring thorough investigation, and eligibility criteria for a couple to be recommended an ART-related procedure like ICSI.

The overall suboptimal investigation of male infertility in clinical practice is a priority gap, given it can further complicate the treatment and management of couples aiming to conceive, especially within a narrow period window. A recent study found that couples tend to have lower quality of life when a male-related factor is at cause compared to exclusively female-related infertility (42). While a literature review conducted by Barratt, Björndahl (43) describes gaps in male infertility research that are yet to be bridged, the current treatment landscape allows HCPs to choose from a variety of treatments relevant to males that can truly optimise chances of conception, including but not limited to varicocele repair, sperm retrieval, transurethral resec-

tion of ejaculatory ducts, microsurgical reconstruction of genital tract, and medical treatments with selective oestrogen receptor modulators, antioxidants and gonadotropins (37, 44-47). This study found that beyond the societal tendency to minimize male contribution to couples' infertility, 10-30% of reproductive urologists and andrologists perceived their knowledge of currently available treatments for males to be less than advanced. These rates were even higher (30-70%) for other specialties (i.e., endocrinology, OBGYN), which tend to focus more on female infertility, but are still expected to demonstrate advanced knowledge in this domain to best serve couples in their reproductive journey. This may explain why almost 20% of physicians involved in ART and male infertility equally report skill gaps for setting realistic expectations with men about their fertility, and chances for conception (48).

In the context of ongoing transmissible viral infections and possible pandemics in the future, which can affect reproductive health, this study found that fertility care professionals perceive counselling patients on vaccines and using telehealth as both relevant skills to the optimal care of patients post COVID-19. This finding supports current efforts to better integrate immunology expertise and research into fertility care, as pregnancy is tied to unique regulatory changes in immunity (49). The skill of counselling patients on the efficacy of vaccines that physicians involved in fertility care report being relevant to their current practice would be most sensible with the support of an enhanced collaboration effort with immunology experts who can best inform patients about the potential risks and benefits of various vaccines on pregnancy and birth outcomes. This type of collaboration could help address patient concerns and health considerations in relation to the use of vaccines during pregnancy, including most recent advances against the SARS-CoV-2 virus (50), which continues to be a relevant health intervention today. In parallel, the introduction of telehealth consultation in fertility clinics appears to have been accelerated by the pandemic, given it offered a solution to stringent physical distancing measures. As found by this study, telemedicine is likely to persist post-pandemic internationally, given it can also be a useful tool to streamline patient monitoring and management via a facilitated access to healthcare professionals (51, 52). However, a meaningful proportion of HCPs who participated in this study perceived their skills as less than advanced for building trust and empathy with patients via telemedicine. Assisting fertility care providers in developing relevant patient-facing skills using virtual communication platforms would be an important next step to ensuring that telehealth continues to be optimally integrated and used as a complement to in-person consultations (53). Studies on this topic are beginning to emerge, and highlight the opportunity to ensure telehealth consultations are carried out in a patient-centric way (54).

Limitations

Despite a total sample size of 76 interview participants and 303 survey participants, given multiple countries were included in this study, the final sample size for the survey did not provide sufficient statistical power to assess significant differences in the identified gaps by country. Hence, only descriptive comparisons could be made. While chi-square analysis assessing variations by regions was possible, a larger representation of countries in each region would have increased the validity of results. The methodology selected for this study was mostly based on self-report. The limitation of self-reporting and recall bias was minimized by a triangulation of sources (i.e., patients, physicians and laboratory specialists) and methods (qualitative and quantitative findings), informed by a literature review at onset of the study (26).

CONCLUSIONS

More efforts need to be placed in ensuring males' aetiologies of infertility are thoroughly investigated in clinical practice and used to inform the appropriate use of ART in a couple's reproductive journey. These challenge areas are priority needs from the perspective of male and female patients, and tailored efforts in CME and CPD for reproductive medicine specialist should be deployed to support physicians and laboratory specialists involved in ART to acquire advanced levels of knowledge and skills to optimally address male infertility factors in a couple's reproductive journey. With the widespread integration

of telemedicine in fertility care and efforts to promote healthy immunity and reproduction in the population, fertility care providers perceive a need to enhance their skills in effectively counselling patients on vaccines and/or using virtual platforms. Since the present study identified barriers at the health-system level, hindering the application of relevant knowledge and skills by HCPs, there is an additional need to better understand the required changes in policies and organizational processes that would facilitate access to andrology services for male infertility and specialized care, as needed.

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COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in this educational and behavioural research study involving human participants were conducted in accordance with the Declaration of Helsinki and national ethical standards (i.e. the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, TCPS). Ethical approval was obtained from IRB Veritas, tracking number: 2021-2618-5696-2, study number: EU-10-001). Informed consent was obtained from all study participants prior to participation in the study.

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Attestation statements

This study and partial results were presented at the American Society for Reproductive Medicine (ASRM) 2022 conference on October 24th and the European Society of Human Reproduction and Embryology (ESHRE) 2023 conference on June 26th (prior to submission). The results presented at both congresses have limited overlap with those presented in the manuscript.

Kupka MS, et al. (2022). "Challenges and potential practice gaps faced by healthcare professionals investigating male infertility: A patient-informed educational study in eight countries." Fertility and Sterility 118(4): e183-e184.

Augustyniak M, et al. (2023). "P-577 Understanding how healthcare professionals address the educational and emotional needs of patients seeking fertility care: an assessment of challenges and barriers across eight countries." Human Reproduction 38. Supplement 1: dead093-123.

Data availability statement

Aggregated and anonymized data will be made available for review or query upon reasonable request.

CONFLICT OF INTEREST

Monica Augustyniak, Patrice Lazure, Sophie Péloquin are employees of AXDEV Group Inc. Sandro C. Esteves declares receipt of speaker's fees from Merck KGaA, Event Planet, and Med.E.A., and unrestricted research grants from Merck KGaA. Giovanni Coticchio, Markus S. Kupka, Chen Hong and Anita Fincham declare that they have no competing interests. Merck KGaA, Darmstadt, Germany reviewed the manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

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Correspondence address:
Sandro C. Esteves, MD, PhD
ANDROFERT, Clínica de Andrologia e Reprodução Humana
Av. Dr. Heitor Penteado, 1464 - Jardim Campinas
Campinas, São Paulo, 13075-460, Brasil
Telephone: +55 19 3295-8877
E-mail: s.esteves@androfert.com.br

APPENDIX

Appendix 1 – Areas of explorations in Phase 1

The following table presents areas of potential challenges and barriers that were explored as part of interviews.

Areas of explorations	PR	LS	Px
Screening & diagnosis of infertility in both men and women	✓	✓	
Integration and use of different genetic testing & screening modalities	✓	✓	✓
Infertility treatment & procedures used for men versus women (e.g., gonadotropins, gamete assessment, selection & retrieval, ovarian stimulation, fertilization, embryo grading/selection, cryopreservation)	✓	✓	✓
Factors influencing treatment selection (including resources, guidelines and patient preferences)	✓		✓
Goals setting, expectations management and assessment/management of patients (both men & women) psychological needs	✓	✓	✓
Monitoring & encouraging treatment adherence (including gonadotropins) via various modalities (e.g., telehealth versus in-person)	✓	✓	✓
Patient-provider communication (including challenging topics of discussion, approach to discussing diagnosis & treatment, emotional support provided)	✓	✓	✓
Telehealth experiences (including factors considered in the selection of patients that would benefit from a telehealth consultations)	✓		✓
Impact of the COVID-19 pandemic on care provided to patient with fertility issues & approaches to support patients in coping with the pandemic	✓	✓	✓
Impact of the COVID-19 pandemic on the diagnosis, treatment & management of patients with fertility issues	✓	✓	✓
Impact of the COVID-19 pandemic on the organization of fertility services & interprofessional work	✓	✓	
Changes brought on by the COVID-19 pandemic expected to remain	✓	✓	✓

PR = physician in reproductive medicine; **LS** = laboratory specialist; **PX** = patient

Appendix 2 – Survey questions, items, responses and recoding for analysis.

Type of gap	Question/item	Responses	Recoding for analysis
Knowledge	To what approximate extent do males contribute to reported cases of infertility globally?	a. Less than 10% b. 10% - 30% c. 31% - 50% d. 50% or more	a or b = Gap c or d = No gap
	For each item listed below, please rate your current level of knowledge based on what is expected of your professional role in the care of patients seeking fertility care. Note: If item is NOT relevant to your current professional role, please select Not relevant ("NR").	NA	NA
	/Eligibility for in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI)	1=No knowledge at all; 2=Basic knowledge; 3=Intermediate knowledge; 4=Advanced knowledge; 5=Expert knowledge NR=Not relevant	1-3 = Gap 4-5 = No gap NR = Excluded from analysis
	/Microsurgical reconstruction of the male genital tract (e.g., vasovasostomy and vasoepididymostomy)		
	/Transurethral resection of ejaculatory ducts		
	/Techniques for varicocele repair		
	/Empirical medical treatment with selective estrogen receptor modulators, antioxidants, and gonadotropin in males		
	/Gonadotropin therapy for hormonal disorders in males		
	/Treatments for male patients that can *improve in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) outcomes		
	/The effectiveness of vaccines available in preventing SARS-CoV-2 infections and limiting symptom severity		
Knowledge	Please rate your level of agreement with each of the following statements. Note: If you are unsure and/or not informed enough to form an opinion, please select "Unsure".	NA	NA
	/Poor embryonic development in an IVF cycle is an indication for ICSI.	1=Strongly disagree; 2=Disagree; 3=Unsure - not informed enough to form an opinion; 4=Agree; 5=Strongly agree	3-5 = Gap 1-2 = No gap
	/With the exception of 0% values (globozoospermia) even very low rates of sperm morphology have poor predictive power.		3-5 = Gap 1-2 = No gap
Skill	For each item listed below, please rate your current level of skill according to what is expected of your professional role in the care of patients seeking fertility care. Note: If item is NOT relevant to your current professional role, please select Not relevant ("NR").	1=No skill at all; 2=Basic skill level; 3=Intermediate skill level; 4=Advanced skill level; 5=Expert skill level NR=Not relevant	1-3 = Gap 4-5 = No gap NR = Excluded from analysis
	/Investigating hypothalamic-pituitary axis dysfunction in males		
	/Investigating spermatogenic defects		
	/Investigating ductal obstruction or dysfunction in males		
	/Investigating infectious disease causes of infertility in males		
	/Investigating systemic causes of infertility in males		
	/Counselling patients on the safety and efficacy of available vaccines (including ones for SARS-CoV-2)		
	/Discussing with patients' evidence regarding the risks versus benefits of SARS-CoV-2 vaccines on pregnancy and birth outcomes		
	/Building rapport with patients via telehealth during an initial evaluation		

Type of gap	Question/item	Responses	Recoding for analysis
Skill (decision-making)	A 45-year-old man and a 32-year-old woman present because they experienced an unsuccessful IVF cycle in another clinic 4 months prior. The previous IVF attempt resulted in 20 mature oocytes and poor embryo development.	NA	NA
	/Which assessments would be obtained to guide further treatment? Check all that apply.	a. Anti-Müllerian hormone b. Anti-sperm antibody titer c. Semen analysis/sperm function test d. Serum testosterone for male e. Serum FSH for male and female f. Genetic tests	a, b, d, e or f = Incorrect c = Correct
	/All results are normal except semen analysis/sperm function test - 3% of sperm with normal morphology. What is the next best course of action?	a. A second cycle of conventional IVF b. Split-cycle IVF/ICSI c. ICSI d. Full andrological evaluation by a specialist	a-c = Incorrect d= Correct
Attitude	Please rate your level of agreement with each of the following statements. Note: If you are unsure and/or not informed enough to form an opinion, please select "Unsure".	NA	NA
	/Investigating the causes of male infertility is simpler than for female infertility.	1=Strongly disagree; 2=Disagree; 3=Unsure - not informed enough to form an opinion; 4=Agree; 5=Strongly agree	4-5 = Gap 1-3 = No gap
Performance	How often do you consider the health of a female patients' male partner when investigating and treating their fertility issues?	a. Always b. Most of the time c. Sometimes d. Rarely e. Never	b-e = Gap a = No gap
System	Has the COVID-19 pandemic resulted in the following changes? If so, what is the likelihood of each change persisting after the COVID-19 pandemic is over?	NA	NA
	/staffing shortages	Part 1. a. Yes b. No Part 2. 1=Very unlikely 2= Unlikely 3= Unsure 4 = Likely 5 = Very likely	Part 1. a = Change b = No change Part 2. 4=5 = Likely or very likely 3 = Unsure 1-2 = Unlikely to very unlikely
	/removal of diagnostic tests for fertility (e.g., fallopian tube examination)		
	/limited access to labs that perform specialised diagnostic testing (e.g., Anti-Müllerian hormone, genetic testing)		
	/limited access to medications needed for fertility treatment		
	/policy changes that required a greater prioritisation of patients for fertility treatment than before		
	/a more conservative approach to preventing ovarian hyperstimulation syndrome and minimise hospitalisation risk		
	/the need to counsel patients on vaccines (including SARS-CoV-2)		
	/an uptake in the use of tools and technologies facilitating communication with patients at a distance		

Appendix 3 - Percentage of HCPs agreeing, unsure or disagreeing with opinion statements.

Statement	Answer	Countries (%, n)								
		South America		Western Europe			Southern Europe		Asia	Mean Total
		BR	MX	DE	FR	UK	IT	SP	CH	Total
Investigating male infertility is simpler than female infertility *	Disagree	54% (21/39)	33% (13/39)	3% (1/37)	37% (14/38)	46% (17/37)	49% (19/39)	44% (17/39)	31% (17/55)	37% (119/323)
	Unsure	0% (0/39)	21% (8/39)	32% (12/37)	29% (11/38)	16% (6/37)	28% (11/39)	21% (8/39)	18% (10/55)	20% (66/323)
	Agree	46% (18/39)	46% (18/39)	65% (24/37)	34% (13/38)	38% (14/37)	23% (9/39)	36% (14/39)	51% (28/55)	43% (139/323)
With the exception of globozoospermia, even very low rates of sperm morphology have poor predictive power *	Disagree	33% (13/39)	21% (8/39)	8% (3/37)	3% (1/38)	22% (8/37)	15% (6/39)	8% (3/39)	11% (6/55)	15% (48/323)
	Unsure	26% (10/39)	33% (13/39)	49% (18/37)	24% (9/38)	24% (9/37)	36% (14/39)	36% (14/39)	22% (12/55)	31% (99/323)
	Agree	41% (16/39)	46% (18/39)	43% (16/37)	74% (28/38)	54% (20/37)	49% (19/39)	56% (22/39)	67% (37/55)	55% (176/323)
Poor embryonic development in an IVF cycle is an indication for ICSI	Disagree	15% (6/39)	18% (7/39)	5% (2/37)	11% (4/38)	22% (8/37)	13% (5/39)	23% (9/39)	13% (7/55)	15% (48/323)
	Unsure	3% (1/39)	26% (10/39)	30% (11/37)	16% (6/38)	35% (13/37)	18% (7/39)	5% (2/39)	31% (17/55)	21% (67/323)
	Agree	82% (32/39)	56% (22/39)	65% (24/37)	74% (28/38)	43% (16/37)	69% (27/39)	72% (28/39)	56% (31/55)	64% (208/323)

BR (Brazil); CH (China); FR (France); DE (Germany); IT (Italy); MX (Mexico); SP (Spain); UK (United Kingdom).
Five-point agreement rating scale was used (1=Strongly disagree, 2=Disagree, 3=Unsure – Not informed enough to form an opinion, 4=Agree, 5=Strongly agree).
*Asymptotic significance (2-sided) < 0.05 for 3x4 (agreement X region) crosstabulation with Pearson Chi-Square statistical test

Appendix 4 - HCP survey responses to a clinical case question.

Question item	Answer	South America		Western Europe			Southern Europe		Asia	Total
		BR	MX	DE	FR	UK	IT	SP	CH	
Which assessments would be obtained to guide further treatment? Check all that apply.	Anti-Müllerian hormone	15% (6/39)	31% (12/39)	70% (26/37)	37% (14/38)	35% (13/37)	28% (11/39)	36% (14/39)	53% (29/55)	39% (125/323)
	Anti-sperm antibody titer	26% (10/39)	28% (11/39)	73% (27/37)	26% (10/38)	41% (15/37)	54% (21/39)	26% (10/39)	76% (42/55)	45% (146/323)
	Semen analysis/sperm function test*	**82% (32/39)**	**82% (32/39)**	**89% (33/37)**	**95% (36/38)**	**76% (28/37)**	**77% (30/39)**	**74% (29/39)**	**91% (50/55)**	**84% (270/323)**
	Serum testosterone for male	26% (10/39)	64% (25/39)	76% (28/37)	24% (9/38)	30% (11/37)	41% (16/39)	28% (11/39)	58% (32/55)	44% (142/323)
	Serum FSH for male and female	21% (8/39)	74% (29/39)	73% (27/37)	45% (17/38)	30% (11/37)	54% (21/39)	33% (13/39)	73% (40/55)	51% (166/323)
	Genetic tests	79% (31/39)	46% (18/39)	35% (13/37)	34% (13/38)	46% (17/37)	38% (15/39)	67% (26/39)	85% (47/55)	56% (180/323)
All results are normal except semen analysis/sperm function test - 3% of sperm with normal morphology. What is the next best course of action?	A second cycle of Conventional IVF	5% (2/39)	13% (5/39)	35% (13/37)	3% (1/38)	8% (3/37)	15% (6/39)	5% (2/39)	7% (4/55)	11% (36/323)
	Split-cycle IVF/ICSI	23% (9/39)	26% (10/39)	8% (3/37)	16% (6/38)	24% (9/37)	15% (6/39)	36% (14/39)	20% (11/55)	21% (68/323)
	ICSI	41% (16/39)	31% (12/39)	41% (15/37)	26% (10/38)	16% (6/37)	26% (10/39)	41% (16/39)	31% (17/55)	35% (113/323)
	Full andrological evaluation by a specialist*	**31% (12/39)**	**26% (10/39)**	**14% (5/37)**	**53% (20/38)**	**16% (6/37)**	**44% (17/39)**	**15% (6/39)**	**40% (22/55)**	**30% (98/323)**
	I do not know	0% (0/39)	5% (2/39)	3% (1/37)	3% (1/38)	5% (2/37)	0% (0/39)	3% (1/39)	2% (1/55)	2% (8/323)

Case: A 45-year-old man and a 32-year-old woman present because they experienced an unsuccessful IVF cycle in another clinic 4 months prior. The previous IVF attempt resulted in 20 mature oocytes and poor embryo development.

***Correct answer**

EXPERT OPINION

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Can CHATGPT provides reliable technical medical information about phimosis?

Edson S. Salvador Junior [1], Carla S. Santos [1], Vimael J. O. Holanda [1], Bruno M. Corrêa [1], Luciano A. Favorito [1]

[1] Unidade de Pesquisa Urogenital, Universidade do Estado do Rio de Janeiro – UERJ, Rio de Janeiro, RJ, Brasil

COMMENT

Phimosis is the most common clinical condition in pediatric urology. Physicians must be trained to answer patients and parent's questions about phimosis and circumcision and make clinical decision based on medical data and patient's medical context. Nowadays, a lot of medical and non-medical source of educational information are disponible for physicians, parents, and patients on internet, such as: Pubmed®, Google Scholar®, Instagram®, Youtube® and chatbots platforms. ChatGPT v3.5 (1) (OpenAI, California, 2022), is a chatbot platform that, according to own ChatGPT definition, is an "Artificial Intelligence system trained on diverse internet text and is capable of answering questions, providing information's and engaging in conversations." (1).

Patients and physicians use ChatGPT as a "search engine" to answer routine medical topics, but the real capacity of answering medical questions in a technical manner and with fidelity to medical established guidelines have been matter of discussion. While some papers say that this tool can provide valuable reliable resources for patients (2-5), other studies draw attention to negative points, such as outdated data, inability to understand psycho-social aspects, lack of transparency, accountability and divergent information's with regard to actual medical literature, with a lot of recommendations against this practice (6-8).

Our objective is to answer the question if ChatGPT can provide reliable technical medical information, consonant with actual medical literature, about phimosis in children for physicians, parents and patients.

Four questions usually made for parents and patients in pediatric urology offices about phimosis were asked to ChatGPT and the answers were compared with the European Association of Urology 2023 recommendation panel guidelines, Pediatric Urology section (2). The answers and guidelines recommendations were analyzed, compared, reviewed and commented by Pediatric Urology Staff of a University Hospital, considered experts in this issue.

QUESTIONS

Question 1: Is topical corticosteroid treatment effective for phimosis?

ChatGPT: Topical corticosteroid treatment can be effective for certain cases of phimosis, particularly when the condition is caused by inflammation or scarring. This type of treatment is often considered before more invasive options like circumcision, especially in cases where the phimosis is not causing significant problems and conservative measures are preferred.

EAU: Conservative treatment of phimosis with topical corticosteroids (ointment or cream) has a high success rate, but surgical treatment may be considered if preferred by caregivers or patients (Level of Evidence 1b). Offer topical corticosteroids (ointment or cream) as first-line treatment in symptomatic phimosis. (Strong rating)

Comments: EAU recommends corticosteroid as a first line treatment, with high success rate, for all patients with phimosis. ChatGPT recommends only for specific cases, and If there is no local complications.

Question 2: Should Balanitis Xerotica Obliterans (BXO) be treated with circumcision in children?

ChatGPT: The management of BXO in children often involves a stepwise approach, and circumcision may be considered in certain cases. However, it's important to note that not all cases of BXO automatically require circumcision. The Treatment options for BXO includes: Topical Corticosteroids, Dilation and Circumcision in cases where conservative measures are not successful.

EAU: Offer circumcision in case of BXO (strong level of recommendation).

Comments: Here are conflicting information. EAU guidelines recommends offer only surgical treatment for these patients without other options, while ChatGPT considers using corticosteroids and preputial dilation.

Question 3: Should we perform circumcision for first time episode of paraphimosis?

ChatGPT: If manual reduction is unsuccessful or if there are recurrent episodes of paraphimosis, a healthcare provider may explore various treatment options. Circumcision may be considered as a preventive measure in some cases, especially if paraphimosis is a recurring problem or if there are underlying conditions contributing to the issue.

EAU: Treat paraphimosis by manual reposition and proceed to surgery if this fails. (Strong level of Recommendation)

Comments: Here there is an agreement between both sources. First-line therapy is manual reposition and surgery is only indicated if there is failure. No regards on EAU recommendations are made for recurrent episodes, while ChatGPT recommends surgical treatment in these cases.

Question 4: What are the main complications of Circumcision?

ChatGPT: Bleeding, Infection, Pain and Discomfort, Swelling and Bruising, Irritation or Injury to the Glans, Inadequate or Excessive skin removal, Complications with anesthesia.

EAU: Complications following circumcision vary and have been reported to be between 0-30%. Non-healing wounds, hemorrhage, wound infection, meatal stenosis, redundant skin, non-satisfying cosmetic appearance and trapped penis may all occur.

Comments: Here there is an almost complete agreement between the two sources, although EAU guidelines provide more detailed information.

DISCUSSION

Actually, internet is the main source of information for physicians, parents and patients. It can provides

technical information for physicians and non-technical for non-healthcare providers. A lot of scientifical effort have been made to explain if ChatGPT can answer technical questions and helps (9, 10) in clinical decision-making process at different areas, such as dermatology (11), surgery (12), emergency medicine (13), urology (14) and other medical areas.

ChatGPT answered questions 1 and 2 differently from classical medical guidelines recommendation, especially in question 2, when formal guidelines recommends surgical treatment, not recommended by the first and it can cause potential damage for the patient if the information provided is used alone. For question 3, there is a partial agreement between the data sources and circumcision is not a formal guideline recommendation after first episode of paraphimosis. In question 4, it appears to have a lot of concordance between both sources, albeit some guideline important topics are not mentioned. In this case both sources of information can be used in a complementary way.

Although ChatGPT can offer good information for general non-healthcare public about the topic "phimosis", there are important conflicts between EAU guidelines and ChatGPT recommendations, especially in guidelines stablished conducts that can lead to wrong medical decision making and potential damage for patients. We do not recommend use of ChatGPT as a technical source of study for physicians and medical decision about phimosis.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Luciano A. Favorito, MD, PhD
Unidade de Pesquisa Urogenital
da Universidade do Estado de Rio de Janeiro - UERJ,
Rio de Janeiro, RJ, Brasil
E-mail: lufavorito@yahoo.com.br

ARTICLE INFO

Luciano A. Favorito
https://orcid.org/0000-0003-1562-6068

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Editorial Comment: Comparative Analysis of Super-Mini Percutaneous Nephrolithotomy and Retrograde Intrarenal Surgery for the Management of Renal Calculi ≤2 cm Among Somali Population

Abdihamid Hassan Hilowle [1], **Abdikarim Hussein Mohamed** [2]

[1] *Department of Urology, Mogadishu Urological Center, Mogadishu, Somalia; 2 Department of Urology, Mogadishu Somalia Turkish Training and Research Hospital, Mogadishu, Somalia*

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Luciano A. Favorito [1, 2]

[1] *Unidade de Pesquisa Urogenital - Universidade do Estado do Rio de Janeiro - Uerj, Rio de Janeiro, RJ, Brasil;* [2] *Serviço de Urologia, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brasil*

COMMENT

The management of the urinary stones constitutes one of the most usual treatments in in urology practice. There are several options for the renal stones treatment: shockwave lithotripsy (SWL), retrograde intrarenal surgery (RIRS) and percutaneous nephrolithotomy (PCNL) (1-3). In the past, PCNL was done for large-volume stones such as complex multiple calyceal calculi, staghorn stones. The reduction of the tract size and the advent of miniaturization of instruments ushered in the development of smaller scopes, smaller retrieval devices, and energy sources were responsible for paradigm shift in the indications for PCNL (3, 4). These miniaturized instruments and accessories obviated the need to dilate the tract beyond 20 Fr.

The newer techniques with Miniperc are suited for stones 1.5–2 cm in size. Microperc and Ultraminiperc may be suitable for stone sizes <1.5 cm (1-4). The present paper aims to comprehensively evaluate the safety and effectiveness of super-mini percutaneous nephrolithotomy (SMP) compared with RIRS (5). The authors studied 210 patients with renal calculi (≤2 cm) undergoing SMP or RIRS, randomly recruited over 4 years and concluded that SMP demonstrated superior efficacy with significantly shorter operating times and reduced hospital stays, suggesting potential advantages for managing lower volume renal stones.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Luciano Alves Favorito, MD, PhD
Rua Professor Gabizo, 104/201 – Tijuca
Rio de Janeiro, RJ, 20271-320, Brazil
Fax: +55 21 38728-802
E-mail: lufavorito@yahoo.com.br

ARTICLE INFO

Luciano A. Favorito
https://orcid.org/0000-0003-1562-6068

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Step-by-step Peritoneal Bladder Flap Bunching (PBFB) technique: an innovative approach following lymph node dissection in robotic radical prostatectomy

Ahmed Gamal [1], Marcio Covas Moschovas [1, 2], Abdel Rahman Jaber [1], Shady Saikali [1], Sumeet Reddy [1], Ela Patel [3], Evan Patel [1], Travis Rogers [1], Vipul Patel [1, 2]

[1] AdventHealth Global Robotics Institute, Celebration, USA; [2] University of Central Florida - UCF, Orlando, USA; [3] Stanford University, Palo Alto, California, USA

ABSTRACT

Introduction: Robot-assisted radical prostatectomy (RARP) has become a popular surgical approach for localized prostate cancer due to its favorable oncological and functional outcomes, as well as lower morbidity. In cases of intermediate- and high-risk prostate cancer, bilateral pelvic lymphadenectomy (PLND) is recommended as an adjunct to RARP (1-3). Despite its benefits, PLND can lead to surgical complications, with postoperative lymphocele formation being the most common. Most postoperative lymphoceles are clinically insignificant with variable incidence, reaching up to 60% of cases 4. However, a small percentage of patients 2-8% may experience symptomatic lymphoceles (SL), which can cause significant morbidity (4, 5).

Surgical technique: We perform our RARP technique with our standard approach in all patients (6). After vesicourethral anastomosis a modified PF created to prevent symptomatic lymphocele. We start by suturing the peritoneal fold on the right side, medially to the vas deferens, followed by a similar stitch on the left side to approximate the edges in the midline. A running suture bunches the bladder peritoneum from both sides, passing through the pubic bone periosteum to secure it in place (7). This approach keeps the lateral pelvic gutters open for lymphatic drainage, while allowing fluid drainage from the true pelvis into the abdomen. A pelvic ultrasound was done for all patients at 6 weeks post operative, and additional clinical follow-up was carried out at 3 months following surgery.

Considerations: We have demonstrated a modified technique of peritoneal flap (PBFB) with an initial decrease in postoperative symptomatic lymphoceles, the technique is feasible, safe, does not add significant morbidity, and does not require a learning curve.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Ahmed Gamal, MD
Adventhealth Global Robotics Institute
380 Celebration place
Orlando, Florida 34747, USA
Telephone: +1 407 403-1271
E-mail: dr.ahmedgamal88@gmail.com

ARTICLE INFO

Ahmed Gamal
https://orcid.org/0000-0002-1708-8534

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Single-Port Robot assisted partial cystectomy for urachal adenocarcinoma

Sij Hemal [1], Sina Sobhani [1], Kevin Hakimi [1], Shilo Rosenberg [1], Inderbir Gill [1]

[1] *Institute of Urology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA*

ABSTRACT

Objective: We present a novel technique to perform single-port (SP) robot-assisted partial cystectomy with excision of the urachal remnant and bilateral pelvic lymph node dissection for urachal adenocarcinoma (1-7).

Materials and Methods: A 41-year-old male presented to the clinic for multiple episodes of hematuria and mucousuria. Office cystoscopy revealed a small solitary tumor at the dome of the bladder, with a diagnostic bladder biopsy revealing a tubule-villous bladder adenoma. Cross-sectional imaging of the chest/abdomen/pelvis revealed a 4.5 cm cystic mass arising from the urachus without evidence of local invasion and metastatic spread. He underwent SP robotic-assisted partial cystectomy with excision of the urachal remnant and bilateral pelvic lymph node dissection. Surgical steps include: 1) peritoneal incision to release the urachus and drop bladder 2) identification of urachal tumor 3) intraoperative live cystoscopic identification of bladder mass and scoring of tumor margins using Toggle Pro feature 4) tumor excision with partial cystectomy 5) cystorrhaphy 6) bilateral pelvic lymph node dissection 7) peritoneal interposition flap to mitigate lymphocele formation.

Results: Surgery was successful, with no intraoperative complications, an operative time of 100 minutes, and estimated blood loss of 20 mL. The patient was discharged on post-op day one, and the Foley catheter removed one week after surgery. Final pathology revealed a 7.5 cm infiltrating urachal muscle-invasive adenocarcinoma of the bladder (pT2b). Negative surgical margins were achieved.

Conclusions: Single-port robot-assisted partial cystectomy for urachal adenocarcinoma is safe and can achieve equivalent oncologic outcomes to the standard of care with minimally invasive and open techniques.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Sij Hemal, MD
Assistant Professor of Clinical Urology
Institute of Urology
USC/Norris Comprehensive Cancer Center, USC
1441 Eastlake Avenue, Suite 7416
Los Angeles, CA 90089, USA
Telephone: +1 323 865-3700
E-mail: kshitij.hemal@med.usc.edu

ARTICLE INFO

Sina Sobhani
https://orcid.org/0000-0001-9089-4148

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