TOWARD BETTER MANAGEMENT OF HYPOSPADIAS: OPTIMIZING ASSESSMENT TOOLS, PRECLINICAL MODELS, AND TISSUE ENGINEERING STRATEGIES

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BY
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DENMARK

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PHD DISSERTATION

by

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CV

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LIST OF MANUSCRIPTS

Clinical articles

- Abbas TO. An objective hypospadias classification system. J Pediatr Urol. 2022 May 11:S1477-5131(22)00193-0.

- Abbas TO, Ali M. Urethral Meatus and Glanular Closure Line: Normal Biometrics and Clinical Significance. Urol J. 2018 Sep 26;15(5):277-279.

- Abbas TO, Vallasciani S, Elawad A, Elifranji M, Leslie B, Elkadhi A, Pippi Salle JL. Plate Objective Scoring Tool (POST); An objective methodology for the assessment of urethral plate in distal hypospadias. J Pediatr Urol. 2020 Oct;16(5):675-682.

- Abbas TO, Ali M. Scrotal base distance: A new key genital measurement in males with hypospadias and cryptorchidism. Curr Urol. 2021 Dec;15(4):214-218.

- Abbas TO. Ultrasonographic Evaluation of the Hypospadiac Penis in Children. Front Pediatr. 2022 Jul 6;10:932201.

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Systematic reviews

- Abbas TO, Braga LH, Spinoit AF, Salle JP. Urethral plate quality assessment and its impact on hypospadias repair outcomes: A systematic review and quality assessment. J Pediatr Urol. 2021 Jun;17(3):316-325.

- Abbas TO, Elawad A, Kareem A, Pullattayil S AK, Ali M, Alnaimi A. Preclinical Experiments for Hypospadias Surgery: Systematic Review and Quality Assessment. Front Pediatr. 2021 Aug 9; 9:718647.

- Abbas TO, Elawad A, Pullattayil S AK, Pennisi CP. Quality of Reporting in Preclinical Urethral Tissue Engineering Studies: A Systematic Review to Assess Adherence to the ARRIVE Guidelines. Animals (Basel). 2021 Aug 21;11(8):2456.

Book chapter

- Abbas TO, De Graaf P, Pennisi CP. The rabbit model in preclinical hypospadias research: strengths and limitations. (Springer) Hypospadiology; Current Challenges and Future Perspectives. (*in press*)

Narrative review

- Abbas TO, Mahdi E, Hasan A, AlAnsari A, Pennisi CP. Current Status of Tissue Engineering in the Management of Severe Hypospadias. Front Pediatr. 2017; 5: 283.

Experimental article

- Abbas TO, Parangusan H, Yalcin, H, Hassan M, Zakrif L, Zandi N, Pennisi, CP. Trilayer chitosan/PLA composite scaffold for urethral reconstruction: evaluation of mechanical, biological, and angiogenic properties (*in preparation*)

PREFACE

This thesis: "***Toward better treatment of hypospadias by improving assessment tools, preclinical models, and tissue engineered grafts"*** has been submitted to the Faculty of Medicine, Aalborg University, Denmark. The experimental work in this thesis has been carried out at the Centre for Advanced Materials, Qatar University, Doha, Qatar, Biomedical Research Centre, Qatar University, Doha, Qatar, Translational Research Centre, Hamad Medical Corporation, Doha, Qatar, Weill-Cornell Medicine-Qatar, Doha, Qatar from September 2017 to February 2022.

During my PhD study, I have been teaching, coordinating teaching, and supervising physicians in Hamad Medical Corporation and Sidra medicine, Doha, Qatar and I have completed PhD courses corresponding to 30 ECTS points. Together these activities correspond to more than a full year of my PhD study.

The thesis is based on several clinical, reviews and experimental studies. The thesis is composed of a general introduction encompassing the topics being explored in the manuscripts, the aim and objectives of the thesis, selected methods are presented, the results are presented as a summary of the manuscripts, a general discussion and finally conclusions and perspectives. The original research articles and the reviews are attached as appendix.

ACKNOWLEDGEMENTS

I would like to thank my wife, Eynas, for granting our family her life. She is compassionate, loving, and kind. When things were challenging, I greatly valued and took heed of your words of encouragement. I am very thankful.

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I would also like to thank my PhD supervisor, Associate Professor Cristian Pablo Pennisi, for his continued support and guidance, and my collaborators for their help and time.

Besides, I want to express special appreciation to the Medical Research Center of Hamad Medical Corporation for funding my related experiments.

LIST OF ABBREVIATIONS

3D	3 dimensional
ALD	AlloDerm
AGD	Anogenital distance
ARRIVE	Animal Research: Reporting of In Vivo Experiments
BAM	Bladder acellular matrix
BCS	Bifurcation of the corpus spongiosum
CAM	Chorioallantoic membrane
CPC	Chitosan/PLA/chitosan
ECM	Extra Cellular Matrix
GMS	Glans-Urethral Meatus-Shaft
HADSCs	Human adipose-derived stem cells
ICC	Intraclass correlation coefficient
MPC	Meatal position-based classification
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
PAA	Peracetic Acid
PC	Penile curvature
PCL	Polycaprolactone
PLA	Polylactic acid
POST	Plate objective scoring tool
PREPARE	Planning Research and Experimental Procedures on Animals: Recommendations for Excellence
PU	Polyurethane
PUG	Penile ultrasonography
SBD	Scrotal Base Distance
SMCs	Smooth muscle cells
SPL	Stretched penile length
TDS	Testicular dysgenesis syndrome
TE	Tissue engineering
TIP	Tubularised incised plate
UDR	Urethral defect ratio
UDS	Undescended testis
UDSC	Urine-derived stem cells
UDT	Undescended testes
UP	Urethral plate

TABLE OF FIGURES

Figure 1. Histological analysis of a cross section through the center of the bulbar urethra is shown in this diagram of the male urinary tract. Histological slides depict the urethra lumen, including the mucosa, submucosa, and spongiosa, as well as the two corpora cavernosa. CC- BY-4.0. from (8).

Figure 2. Different types of hypospadias. The meatus is located at an abnormal position. CC- SA-1.0.

Figure 3. Autologous sources of grafts for urethral replacement.

Figure 4. Grafting of urethra using acellular matrices (cross sections). The justification for onlay and inlay procedures. A. The distance between the native urethra and the graft must be less than 1.5 cm (distance X–Y), and is typically less than 1 cm. The arrows represent the direction of cellular ingrowth." B. According to this rule, regrown urethral tissue is healthy. With permission from Elsevier From (49).

Figure 5. Limitations of the current hypospadiology related disciplines, objectives of the PhD projects and studies conducted.

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Figure 10. A new objective algorithm for the management of hypospadias based on the outcomes of the three key clinical studies to optimize the hypospadias evaluation tools.

ENGLISH SUMMARY

Hypospadias is a congenital malformation of the male external genitalia in which the meatal opening is located at the ventral aspect of the penis, affecting approximately 1 in 125 boys. There are a variety of surgical treatments for hypospadias, where the aim is to position the urethral opening at the tip of the glans and straighten the penis. Urethroplasty treatments usually involve the use of grafts, which may consist of the native urethral plate, autologous tissue, or tissue engineered constructs. The development of improved grafts for the treatment of hypospadias involves preclinical testing in animal models. With the goal of addressing some of the current challenges in the management of hypospadias, a series of studies were conducted in this PhD work.

The first series of studies focused on standardizing the evaluation of hypospadias abnormalities. Objective and reproducible tools for evaluating the severity of hypospadias anomaly were proposed, including methods to assess the quality of the urethral plate and to measure the degree of associated penile curvature. These methods utilized noninvasive imaging techniques based on ultrasound and state-of-the-art artificial intelligence. The studies have led to the development and validation of objective tools to assess these anatomic variables, which may be useful in personalized patient care and future research evaluating the outcomes of hypospadias treatment.

The second series of studies focused on the development and fabrication of grafts using tissue engineering concepts. Acellular scaffolds consisting of a three-layer structure of poly(lactic acid) and chitosan nanofibers were developed and tested in vitro. The scaffolds exhibited better water absorption, mechanical and biological properties compared to single-layer structures. In perspective, the proposed scaffolds appear to support the microenvironmental needs of different urethral cell types and adequate mechanical integrity, which should promote urethral formation in vivo.

Finally, the third series of studies focused on evaluating the advantages and limitations of the rabbit model currently used in the development of urethroplasty approaches. Guidelines from ARRIVE were used to generate systematic reviews that were helpful in identifying critical aspects of preclinical research that were not adequately addressed in the published studies. Considering the limitations identified, a paradigm for urethroplasty research was proposed that may help improve future research in this field.

DANSK RESUME

Hypospadias er en medfødt misdannelse af den mandlige eksterne kønsorganer, hvor den meatale åbning er placeret på den ventrale del af penis og påvirker ca. 1 ud af 125 drenge. Der er en række kirurgiske behandlinger for hypospadias, hvor målet er at placere urinrørets åbning på spidsen af glans og rette penis. Urethroplasty-behandlinger involverer normalt brugen af grafts, som kan bestå af den native urethrale plade, autologt væv eller vævskonstruktioner. Udviklingen af forbedrede grafts til behandling af hypospadias involverer prækliniske test i dyremodeller. Med målet om at tackle nogle af de nuværende udfordringer ved behandling af hypospadias blev en række studier gennemført i dette Ph.D.-arbejde.

Den første række af studier fokuserede på at standardisere evalueringen af hypospadias-abnormaliteter. Objektive og reproducerbare værktøjer til vurdering af alvorligheden af hypospadias-anomali blev foreslået, herunder metoder til at vurdere kvaliteten af den urethrale plade og til at måle graden af tilknyttet penis krumning. Disse metoder anvendte ikke-invasive billedteknikker baseret på ultralyd og state-of-the-art kunstig intelligens. Studierne har ført til udviklingen og valideringen af objektive værktøjer til at vurdere disse anatomi-variabler, som kan være nyttige i personlig patientpleje og fremtidig forskning i evalueringen af resultaterne af hypospadias-behandling.

Den anden række af studier fokuserede på udvikling og fremstilling af grafts ved hjælp af koncepter inden for vævsteknologi. Acellulære støttestrukturer bestående af en trelagsstruktur af poly (mælkesyre) og chitosannanofibre blev udviklet og testet in vitro. Støttestrukturerne viste bedre vandabsorption, mekaniske og biologiske egenskaber sammenlignet med enkeltlagsstrukturer. Set i perspektiv ser de foreslåede støttestrukturer ud til at støtte mikromiljøbehovene hos forskellige urethrale celletyper og tilstrækkelig mekanisk integritet, hvilket bør fremme urethral dannelse in vivo.

Endelig fokuserede den tredje række af studier på at evaluere fordelene og begrænsningerne ved kaninmodellen, der i øjeblikket anvendes i udviklingen af urethroplasty-tilgange. Retningslinjer fra ARRIVE blev brugt til at generere systematiske anmeldelser, som var nyttige til at identificere kritiske aspekter af præklinisk forskning, som ikke blev tilstrækkeligt adresseret i de offentliggjorte studier. Med hensyn til de identificerede begrænsninger blev der foreslået et paradigme for forskning i urethroplasty, som kan være med til at forbedre fremtidig forskning på dette område.

Chapter 1: Introduction

As a pediatric urologist, I treat clinical conditions of the urinary tract in children. The most difficult instances I have encountered are those in which subjective terminology typically employed to describe hypospadias abnormality or its linked effects impedes my ability to make exact decisions. Frequently, we also struggle to manage patients with prior surgical operations who present with problems, especially in the absence of tissue. In addition, I have encountered challenges while analyzing and comprehending preclinical animal studies on hypospadias.

Hypospadias is the most common penis malformation, affecting around one in every 125 boys. Multiple investigations over the past three decades have revealed an increase in incidence, although the explanation is uncertain. (1–3) Given its prevalence, hypospadias can place a significant burden on medical resources. (4) Furthermore, most of the literature on hypospadias lacks objective descriptions of the principal anatomical features associated with the condition, and the results of preclinical animal studies are difficult to translate. Nevertheless, there are occasionally limitations to the present therapeutic options, and the feasibility of the treatment can be hindered by a shortage of tissue and donor site morbidity. These individuals may benefit from alternative treatments and enhanced outcomes made possible by tissue engineering.

Given the limitations in the current diagnosis and treatment of hypospadias, the overall goal of this Ph.D. dissertation is to improve the treatment of hypospadias by means of optimizing assessment tools, preclinical models, and tissue engineering strategies. Within this broad goal, the dissertation includes developing tools to improve objectivity in describing the anatomical elements associated with hypospadias anomaly, developing and testing a synthetic scaffold with angiogenic potential, and discussing how to improve the design and reporting of preclinical hypospadias studies.

Chapter 2: Background

2.1 Structure and function of the male urethra

The urethra is a conduit that connects the bladder to the tip of the penis and has the primary purpose of transporting urine and sperm from the bladder to the outside of the body. The male urethra can be split into three major segments: the prostatic part, which passes through the prostate, the membranous urethra, which is covered by the urogenital diaphragm, and the bulbar urethra, which is surrounded by a highly vascular erectile tissue known as the spongiosum. **(Figure 1)** Each of the three layers of the urethra, the mucosa, submucosa, and spongiosum, has its own extracellular matrix (ECM) composed of collagens, glycosaminoglycans (GAGs), and elastin. (5,6). Urothelium in a healthy urethra functions as a barrier to prevent urine from leaking into deeper tissues; this function is maintained by three structures: uroplakin proteins in the apical cell membrane, tight junctions between the superficial umbrella cells, and urothelial GAGs and proteoglycans covering the umbrella cells. (7)

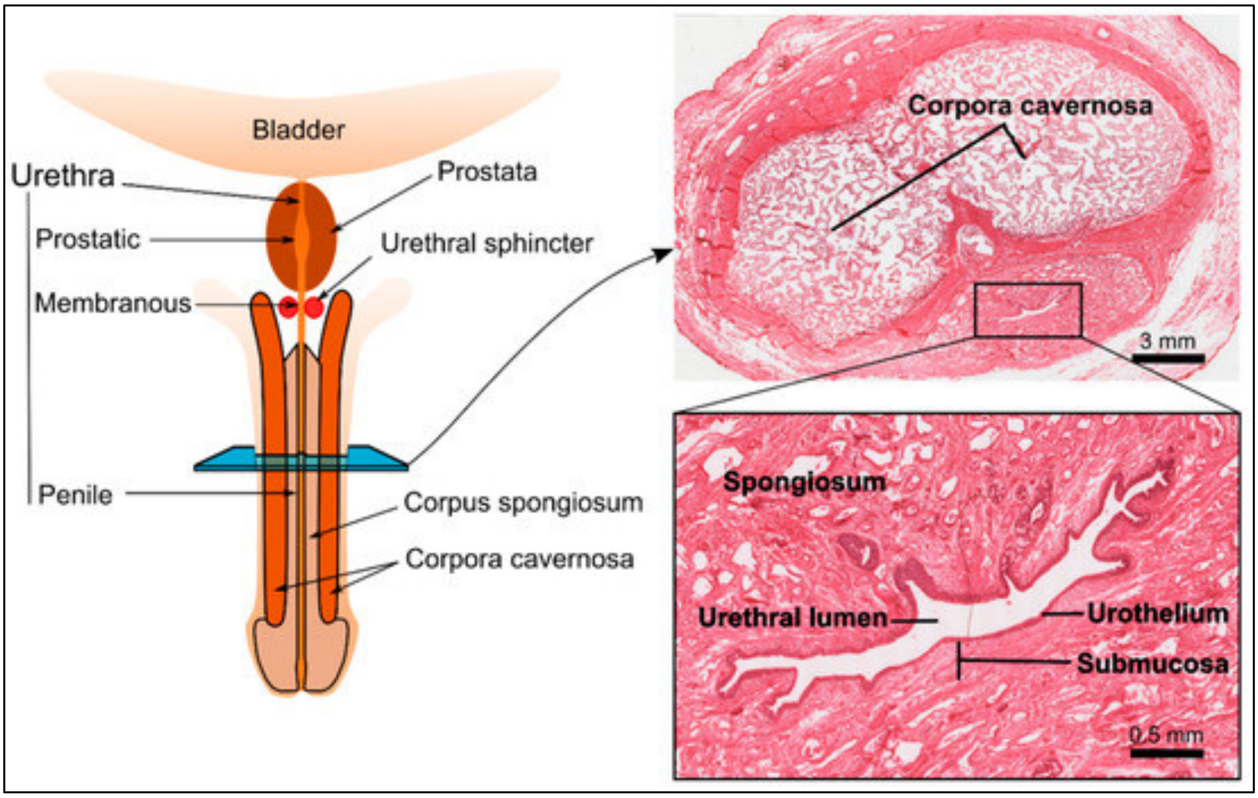

Figure 1. Histological analysis of a cross section through the center of the bulbar urethra is shown in this diagram of the male urinary tract. Histological slides depict the urethra lumen, including the mucosa, submucosa, and spongiosum, as well as the two corpora cavernosa. CC- BY-4.0. from (8).

2.2 Hypospadias anomaly and its management

Hypospadias is a congenital malformation of the male external genitalia in which the meatal opening is improperly situated on the ventral (lower) part of the penis. The urethral aperture may be located anywhere between the glans and proximally toward the scrotum or perineum. In the more severe (proximal) forms, it is also related with penile curvature. Hypospadias is the most prevalent penis deformity, affecting around 1 in 125 boys. Multiple studies have demonstrated a rise in incidence during the past three decades, but the cause is unknown. (1–3)

There are numerous classification systems for hypospadias, but the most commonly used one was created by Barcat and revised by Duckett. In this classification system, the severity of hypospadias depends primarily on the urethral meatal opening, with anterior hypospadias (distal to the subcorona) being the least severe, followed by middle hypospadias (on the middle of the penile shaft), and posterior hypospadias being the most severe (penoscrotal to perineal). (9) (**Figure 2**)

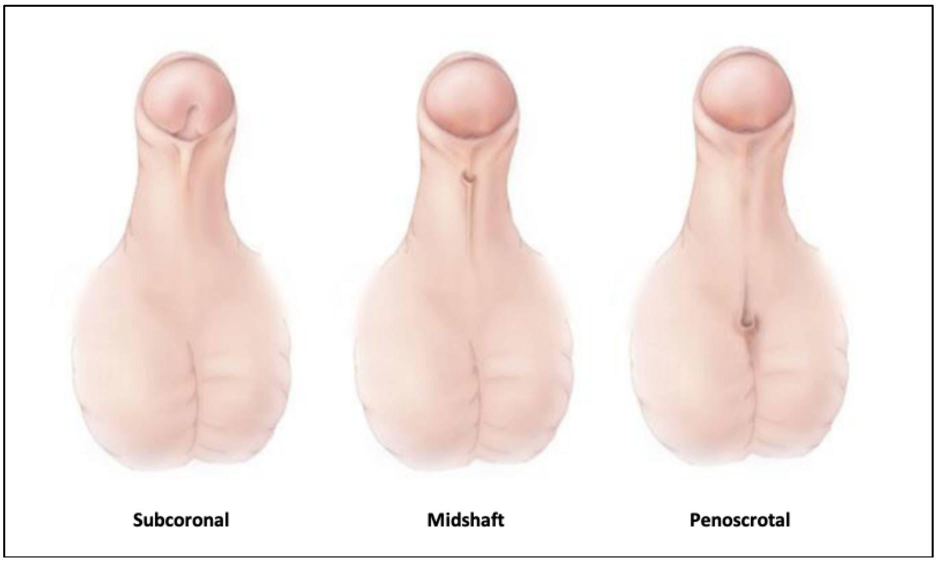

Figure 2. Different types of hypospadias. The meatus is located at an abnormal position. CC- SA-1.0.

Although about 300 options exist for hypospadias repair, the effectiveness of the procedure depends primarily on the surgeon's expertise and the availability of sufficient tissue. In recent decades, however, there have been major advances in the treatment of hypospadias. (9) The ultimate goal of the various surgical procedures is to achieve a straight penis and urethral meatus at the tip of the glans through the tubularization of suitable tissue. (10) These tissues may be the original urethral plate, a graft harvested from an ectopic region, or even synthetic. (11) For distal hypospadias, however, the tubularization of the orthotopic urethral plate with or without incision is currently the most common treatment method. In the 19th century, Thiersch and Duplay described for the first time the tubularization of an intact urethral plate. (12) The tubularization originally terminated at the coronal sulcus, (13) then progressed distally to the proximal glans penis, (14) and finally reached the glans tip. But tubularization of the intact plate has not been widely used because it works best when the urethral plate is both wide and deep, which does not happen very often. (15,16)

Due to the wide range of anatomic phenotypes associated with hypospadias, no single urethroplasty may be suitable for all patients. A more severe phenotype is typically associated with an increased risk of postoperative complications. In addition to fistulas and urethral strictures, complications can also include dehiscence of the repair, recurrence, or persistence of the chordee. Unfortunately, unsuccessful hypospadias repair can necessitate multiple corrective surgical procedures. In contrast, patients with severe hypospadias frequently require additional tissue to reconstruct the urethra. **(Figure 3)** A pedicle flap, which is secured from the internal portion of the dorsal prepuce and then transferred ventrally, is one of the preferred surgical options for some urologists (onlay approach). The urethral plate is utilized as a covering plate and forms the top of the neourethra in this technique. The rectangle of pedicle preputial mucosa flap is then sewn with fine absorbable sutures along the rims of the urethral plate (6-0 or 7-0 polydioxanone or polyglactin). Its complications include fistulas in 5–10% of patients, meatal stenosis in 1%, and urethrocele (i.e. diverticulum) in 0.5%, (17) although others report a different prevalence of complications in 42% of patients, including breakdowns in 7%, fistulas in 23%, urethral strictures in 9%, and diverticula in 3%; it should therefore be avoided whenever possible. (18)

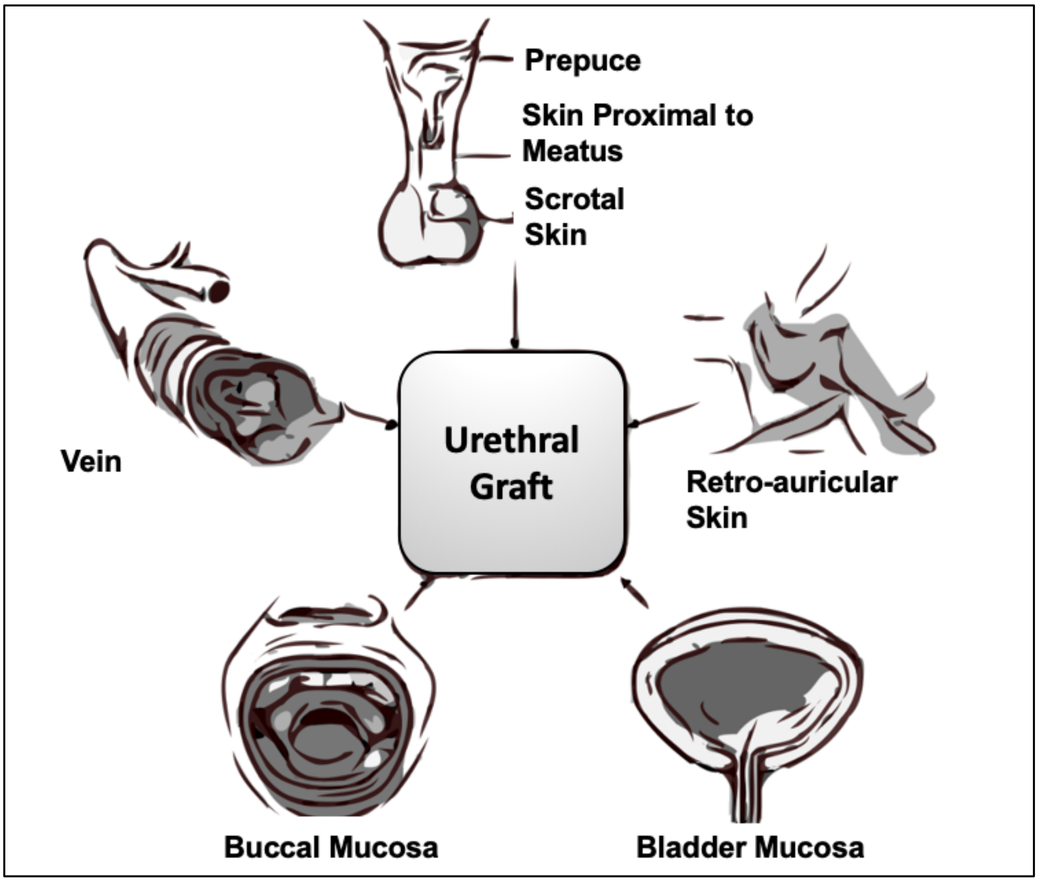

Figure 3. Autologous sources of grafts for urethral replacement.

This was followed by the development of another surgical technique: the tubularised-incised plate (TIP) approach by Snodgrass et al., which has also been performed on patients with proximal hypospadias without severe penile curvatures (which sounds a bit contradictory) while the urethral plate is flexible. A study of 33 patients with recurrent hypospadias revealed a 33% incidence of complications, of which 21% were fistulas. (19) In addition, a meta-analysis revealed that the rates of complications in the various studies utilizing the same TIP repair varied significantly, ranging from 11.1% to 33.3%, with an average of 20.7%. (20) Alternatively, a Snodgraft (using Snodgrass technique "TIP" with the addition of an Inlay graft) is likely used to preserve the urethral plate's character, as depicted above. In a group of people who had dorsal onlay graft urethroplasty in 2002 and were followed up for a long time (21.5 to 43 months), the success rate went from 92% to 85%. (21)

Free grafts are an alternative technique for reconstructing the defective urethral plate. One of the graft sources was skin, which had a poor track record as a substitute for the urethra, particularly extra-genital skin. In contrast, bladder mucosa grafts enjoyed a period of popularity in both primary and revision procedures. In 66% of the cases, additional procedures were required due to the occurrence of complications, including meatal stenosis and urethral prolapse. Currently, the most common method is a free graft of buccal mucosa taken from the inside of the cheek or the lower lip.

Bracka, on the other hand, reported better cosmetic outcomes using two-stage procedures, which were inspired by Cloutier's technique in 1962. (22) In his personal series, Bracka found fistulas in 5.7% of cases and strictures in 7%. The primary step of repair involves a wide dissection of the ventral portion of the penis, from the hypospadiac meatus to the glans, which is typically opened. A rectangle of inner prepuce or buccal mucosa is adhered to the ventral portion of the penis and secured with a "tie-over" weight dressing. Six months later, the urethral plate that was built in the first step is turned into tubes in the second step of the repair. Buccal mucosa grafts yielded success rates of 81% in a single-stage technique and 82.3% in a multistage technique, (23) whereas penile skin grafts yielded success rates of 80%. In addition to donor site bleeding, infection, pain, parotid duct injury, graft contracture, and numbness, the harvesting of buccal mucosa grafts has its own peculiar complications. (24,25)

The success of hypospadias treatment is determined in part by evaluators' subjective impressions of the severity of the condition, the quality of the urethral plate, and the degree of related penile curvature. Future studies evaluating hypospadias treatment outcomes may benefit from the development of standardized objective techniques to quantify these anatomic factors.

2.3 Role of tissue engineering in urethral replacement

The complexity of the surgical procedures involved in treating proximal hypospadias, as well as the risk of infection at the graft donor site, both contribute to the fact that the current methods for treating proximal hypospadias have a number of downsides. In addition, the rates of reoperation and complications continue to be unacceptably high. Due to these restrictions, there has been an increase in research into tissue engineering technologies, which could provide an option that is more suitable for the restoration of urethral function in children.

Tissue engineering combines the fields of cell transplantation and materials engineering in an effort to find a suitable replacement or repair for damaged human tissue. (26) In the past few decades, the evolution of biomaterial development has progressed through several generations. For medical purposes, natural or synthetic biomaterials are fabricated for implantation in biological environments to serve a specific function. (27) Some examples of biomaterials that have been used for tissue engineering of the urethra are summarized in **Table 1.** These materials must be biocompatible, i.e., capable of performing their intended function with a minimally controlled host reaction. (28) The majority of scaffolds are intended to be biodegradable with the goal that cells introduced into the scaffolds will form new tissue and support tissue matrix, replacing the implanted scaffold as it degrades for a successful long-term repair. Additionally, biodegradable implants eliminate the need for a second surgical procedure to remove them. (29) The design requirements of the scaffold which appear crucial to mimic the structural, physical, and biological properties of the native urethra are reviewed in (8).

Table 1: Some examples of tissue engineering approaches for treatment of male urethral strictures

Type of approach	Graft material	Type of study	Ref.
Acellular scaffolds	Bladder acellular matrix	Repair of urethral defects in rabbits	(30)
	Collagen-based inert matrix from bladder submucosa	Hypospadias correction	(31)
	Acellular urethral matrix	Repair of urethral defects in rabbits	(32)
Cell-laden scaffolds	SIS seeded with urine-derived stem cells	Repair of urethral defects in rabbits	(33)
	Polylactid acid seeded with Rabbit adipose-derived stem cells	Repair of urethral defects in rabbits	(34)
	In vitro cultured urothelial cells on acellular dermis	Hypospadias correction	(35)

The ideal tissue engineered construct for urethral replacement possesses biocompatibility, the capacity to be well vascularized, and biodegradability, if possible. (36,37) To obtain a fully differentiated and functional urethra, the degradation process must coincide with the regeneration timeframe of the local surrounding tissues. (38) This material should also be flexible enough to accommodate the urine jets produced during urination. It is possible to adjust its compliance by manipulating its mechanical properties to achieve the optimal stretchability. (39) Additionally, the structure must be impermeable to urine, as urine is cytotoxic to the surrounding tissues. (40) Scaffolds are used in tissue engineering to facilitate new cell proliferation, direct the placement of substitute tissues, and modulate their final shape. Therefore, the perfect scaffold should have several features, including being similar to extracellular matrix (ECM) in function and structure, being biocompatible and degradable in a controlled manner matching the growth of the target tissue for replacement with nontoxic degradation products, and being simple to manufacture. (41) Electrospinning (ES) is the most often employed biomaterials production technology due to its superior control over fiber orientation, size, and porosity. (42,43) It can generate very porous ECM scaffolds. Numerous spinning factors, including voltage, distance, solution type, and concentration, among others, are modifiable in order to obtain results matched to specific requirements.

On the potential beneficial effects of cell seeding of tissue-engineered scaffolds for the urogenital system, there is currently no consensus. Animal studies have shown that reconstructing the urethra with various acellular natural and synthetic materials is safe and effective. (44) It was discovered that natural biomaterials, such as small intestinal submucosa, bladder acellular mucosa, acellular dermis, and urethral acellular matrix, are clinically safe and have a success rate of greater than 75% in the majority of cases. The maximum defect distance suitable for normal tissue formation using acellular grafts that rely on native cells for tissue regeneration appears to be less than one centimeter (44). Consequently, the size of the defect to be repaired may restrict the indications for using acellular matrices in tubularized grafts. (45) If the area to be replaced is small in at least one dimension and an onlay graft can be utilized, cells are not required for proper healing to occur. (45) Acellular substances have not been effective for tubularized urethral repairs (46). To overcome the limitations of acellular materials currently used for urinary tract reconstruction, additional refinements are needed. Despite numerous animal studies, few clinical studies of cell-based TE substitutes for urethral reconstruction in hypospadias and stricture have been published and demonstrate the feasibility and efficacy of using TE-based approaches to treat urethral disease. (47) However, some argue that cells are necessary for urethral repair in constructs longer than 0.5 cm. (45) **(Figure 4)** Cells required to seed the scaffold can be extracted either invasively or noninvasively. The disadvantages of invasive procedures (such as open bladder biopsies) are that they frequently collect an insufficient number of cells and require general anesthesia. In addition, the morbidity associated with the donor site can pose significant risks, such as bleeding and infection. In contrast, several non-invasive techniques have been utilized to collect the necessary cells. For example, bladder washings have been shown to be a safe, highly reproducible, and practical method for obtaining urothelial cells from adults and children. (48) However, since cellularizing grafts prior to implantation presents significant challenges, the production of such an acellular graft material may facilitate the process of tissue-engineered urethral replacement.

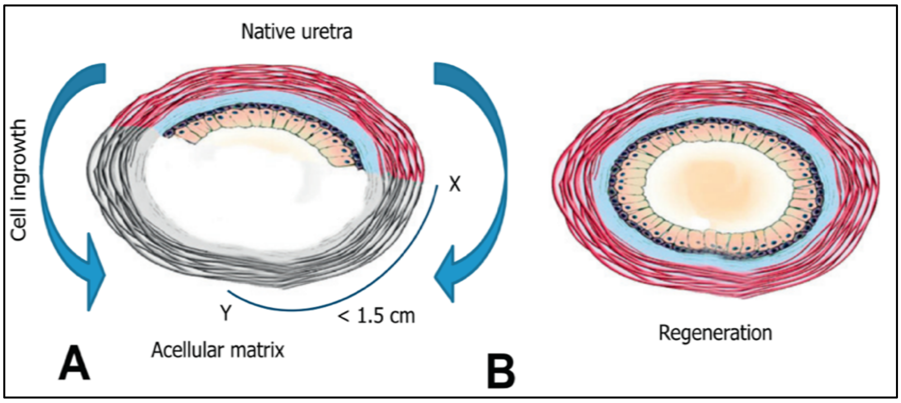

Figure 4. Grafting of urethra using acellular matrices (cross sections). The justification for onlay and inlay procedures. A. The distance between the native urethra and the graft must be less than 1.5 cm (distance X–Y), and is typically less than 1 cm. The arrows represent the direction of cellular ingrowth." B. According to this rule, regrown urethral tissue is healthy. Reproduced from (49) with permission from Elsevier.

2.4 Urethral experiments animal models

In order to conduct in vivo testing, it is necessary to select an animal model (50,51) with a similar histology and general conformation to the human penile urethra. Functionally and structurally, animal models are simplified representations or reproductions of the original system of interest. In comparison to experimentation in humans, animal models are frequently easier to manipulate and control, and ethical problems may be less challenging. In general, the selection of an animal model is reliant on the investigator's experience, ethical sensitivity, and practical limits, as well as budgetary factors, despite the availability of other, more translatable models (52).

A superior animal for urethral tissue engineering and reconstruction has not been established to our knowledge, although numerous animal models have been employed in the past (44,53). According to three recent systematic reviews, (24,44,54) rabbits were most commonly used in urethral tissue engineering research due to the ease of access to the urethra (55) and the histological and functional similarity between the rabbit and human urethra. However, the anatomy of rabbits has several limitations. (56,57) For instance, in severe hypospadias, there is frequently a significant ventral penile skin deficiency that necessitates the use of skin flaps that lack the same thickness and vascularity, whereas in rabbits, the ventral aspect of the penis is supported by the perineal peri-anal area when the skin web between these structures is reconstructed. (58)

Chapter 3: Rationale and aims

3.1 Diagnosis and management of hypospadias

Despite the importance of evaluation of the key anatomical variables in hypospadias cases including the degree of hypospadias, the quality of the urethral plate, and the degree of associated penile curvature in determining the success of hypospadias correction, these evaluations remain subjective. Creation of standardized objective tools to measure these elements, could help in future research on hypospadias treatment outcomes evaluation. Therefore, one of the goals of this thesis was to develop an objective and reproducible tool for the qualification of the urethral plate. On the other hand, a recent review revealed that the currently utilized tools for the measurement of the degree of penile curvature associated with hypospadias display significant inter- and intra- observer discrepancies.(59) These tools include visual inspection, goniometer, standard geometric mobile applications. This was a trigger to create a more precise approach for the measurement of penile curvature and explore the potential of artificial intelligence to further improve its precision, and functionality (**Figure 5).** Furthermore, most hypospadias evaluation and its anatomical variables' categorization tools are subjective. Various methods, like histological staining (60), elastography (61), and magnetic resonance imaging (62), were employed to evaluate the anatomy of hypospadiac penis. Ultrasonography is a valuable diagnostic imaging method because to its non-ionizing feature and multiplanar capabilities, notwithstanding the radiation impact to the gonads (particularly as many affected patients are children or young adults). Using additional noninvasive imaging techniques, such as ultrasonography and cutting-edge artificial intelligence, could help eliminate inter- and intra-observer variability and improve objectivity and reproducibility.

3.2 Tissue engineered urethral grafts

Current methods for treating proximal hypospadias have a number of drawbacks that stem from the severity of the surgical procedures involved and the risk of scarring at the graft donor site. Reoperation and complication rates also remain unacceptably high. These limitations have prompted research into tissue engineering methods, which may provide an improved option for urethral restoration in children (**Figure 5**). Scaffold materials that might meet the clinical performance requirements are still being sought for, despite the extensive investigation into a wide range of degradable biomaterials such decellularized matrices, natural and synthetic polymers. Therefore, it was postulated that the use of acellular trilayer scaffolds in urethral applications, could regenerate the 3D architecture, the microenvironmental requirements of the different urethral cell types, and the diffusion barrier function and structural integrity.

3.3 Preclinical urethral experiments

In the disciplines of hypospadias surgery and urethral tissue engineering, preclinical research has not yet yielded an appropriate therapy option for patients. This gap may be owing in part to inadequate study design and reporting. In addition, although numerous animal models have been utilized in prior research, none of them are currently regarded as definitively superior to the others. Recent comprehensive reviews indicate that rabbits are the most widely used model for urethral research. The aim was to examine the benefits and drawbacks of adopting the rabbit animal model for a urethroplasty investigation, with an emphasis on the experimental design limitations. In addition, ARRIVE was used to identify gaps in study reporting and to suggest a framework for urethroplasty studies. (**Figure 5)**

In order to overcome the above-mentioned limitations, the Ph.D. project has the following objectives:

1. To assess the limitations of currently utilized evaluation tools of hypospadias classification, quality of the urethral plate and penile curvature.
2. To propose and validate new objective tools for the evaluation of hypospadias severity and its associated anatomical variables.
3. To fabricate acellular scaffolds that could regenerate the 3D architecture and the microenvironmental requirements of the urethra.
4. To appraise the quality of reporting of preclinical urethral experiments
5. To propose a standardization scheme to be used when designing preclinical studies of urethroplasty, identifying the most important factors to characterize.

The following five chapters (Chapters 4 to 8) contain the studies carried out to achieve the first two objectives of the thesis. The third objective is addressed by a study presented in Chapter 9, while the fourth and fifth objectives are addressed by the studies presented in Chapter 9 of the thesis.

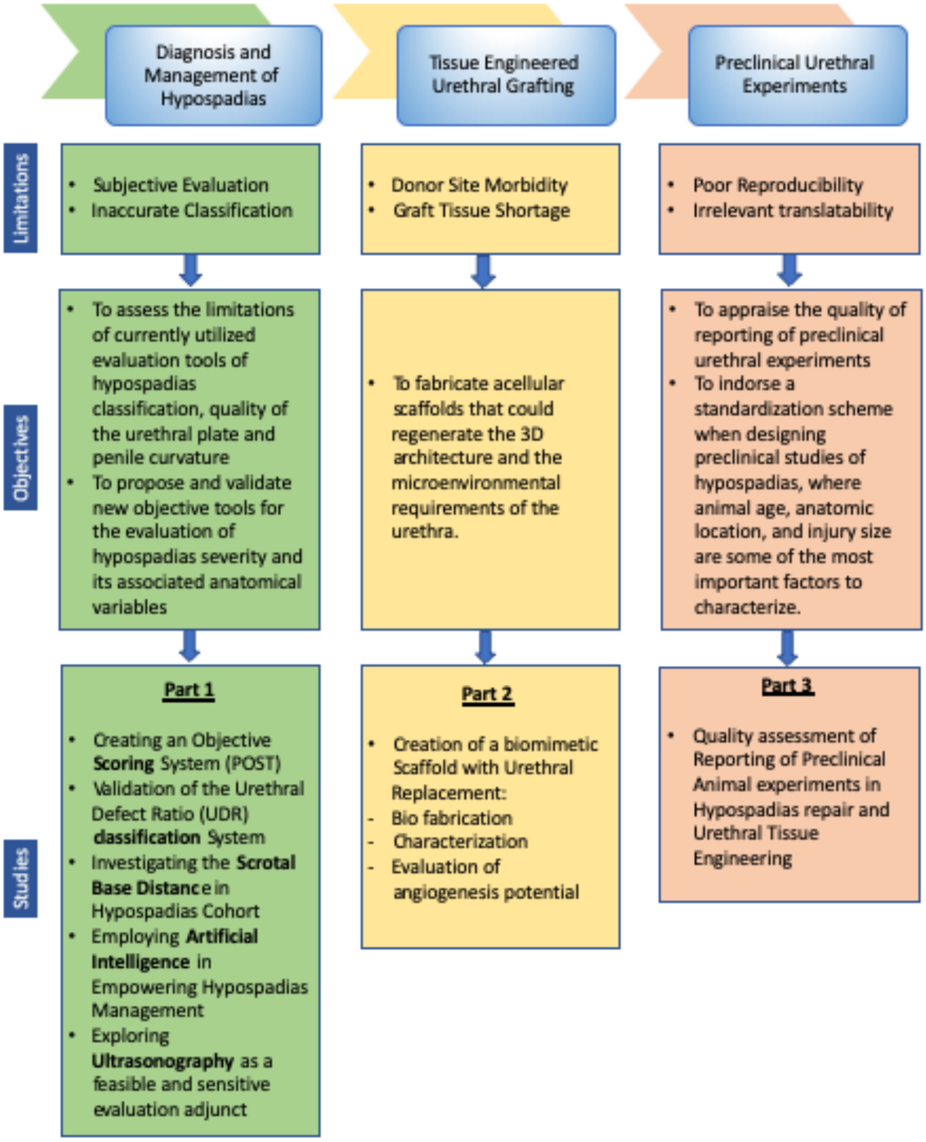

Figure 5. Limitations of the current hypospadiology related disciplines, objectives of the PhD projects and studies conducted.

Chapter 4: Design and validation of the urethral defect ratio hypospadias classification system

This chapter summarizes the results and main conclusions of the study published in the following paper:

- Abbas TO. An objective hypospadias classification system. J Pediatr Urol. 2022 May 11:S1477-5131(22)00193-0. doi: 10.1016/j.jpurol.2022.05.001. PMID: 35644790. (Appendix; Page 1)

4.1 Introduction

The severity of the disease and the available options of the surgical repair depend on the degree of hypospadias present. Consequently, many different categorization systems have been devised to classify the severity of hypospadias depending on the location of the urethral meatus. However, the true location of spongiosal bifurcation is not reliably considered by these classification schemes. Furthermore, it was known for some time that external hypospadiac meatal location does not reliably indicate severity or complexity of the surgical correction required. (63,64) A multivariate study showed that meatal site does not predict incidence of post-operative problems. (65) It was aimed to create an objective hypospadias classification system that can overcome these limitations.

4.2 Methods

For this purpose, primary hypospadias patients younger than 18 years old were included. Extent of urethral defect (distance between glandular knobs and bifurcation of the corpus spongiosum (BCS)) was divided by stretched penile length (SPL) to determine urethral defect ratio (UDR). **(Figure 6)** Thereafter, the severity of hypospadias was divided into three classes (UDR <0.5, 0.5-0.99, and >1.0). As a means of measuring the consistency of ratings for the UDR across reviewers, the ICC was calculated. Moreover, UDR ratios were analyzed using linear regression in order to determine whether they were more strongly correlated with urethral plate quality or the degrees of curvature. **(Figure 6)**

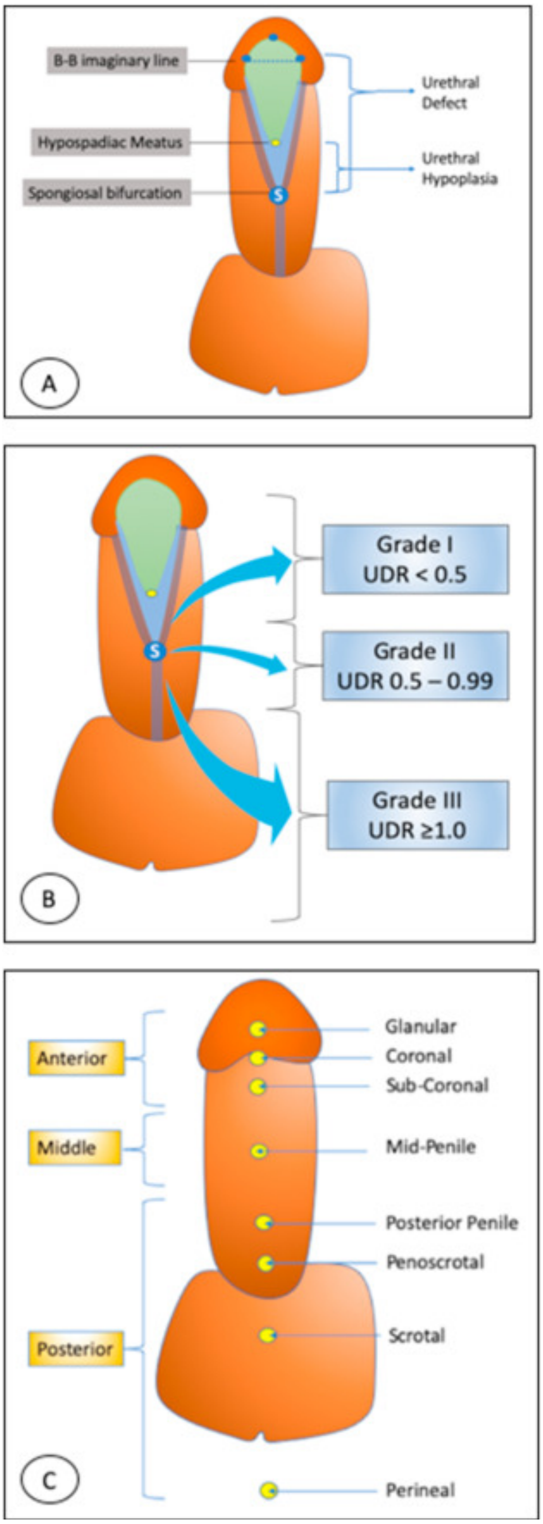

Figure 6. A) The new UDR-based classification (UDC) system for the severity of hypospadias, displaying the key anatomical definitions studied; and B) the grades of hypospadias severity. (C) Meatal position-based classification (MPC) types of hypospadias (Duckett 1989 (66)).

4.3 Key results

A total of 67 patients with a mean age of 12.3 ± 3.7 months were included. UDR ranged from 0.2 and 1.3. Hypospadias severity varied significantly between UDR grades, which corresponded with degree of curvature (P 0.0001), urethral plate quality (P 0.0001), and associated malformations (P 0.05). The ICC value of 0.998 (95% CI: 0.998, 0.9999) was obtained to analyze the intra- and inter-rater agreement of the two reviewers in UDC system.

4.4 Conclusions

The UDR hypospadias classification system appears to represent an objective, practical, and reliable technique for measuring the severity of hypospadias that considers the degree to which the urethra is underdeveloped in relation to the penile shaft. This proposed system can help surgeons more accurately communicate with one another and enable accurate descriptions of hypospadias-spectrum defects. These findings demonstrate that the reviewers have been highly consistent and reliable in their interpretations across multiple readings.

Chapter 5: Creation of scoring system for the objective quantification of the urethral plate

This chapter summarizes the results and main conclusions of the study published in the following papers:

- Abbas TO, Braga LH, Spinoit AF, Salle JP. Urethral plate quality assessment and its impact on hypospadias repair outcomes: A systematic review and quality assessment. J Pediatr Urol. 2021 Jun;17(3):316-325. (Appendix; Page 9)
- Abbas TO, Ali M. Urethral Meatus and Glanular Closure Line: Normal Biometrics and Clinical Significance. Urol J. 2018 Sep 26;15(5):277-279. (Appendix; Page 19)
- Abbas TO, Vallasciani S, Elawad A, Elifranji M, Leslie B, Elkadhi A, Pippi Salle JL. Plate Objective Scoring Tool (POST); An objective methodology for the assessment of urethral plate in distal hypospadias. J Pediatr Urol. 2020 Oct;16(5):675-682. (Appendix; Page 22)

5.1 Introduction

Different surgical procedures for hypospadias repair have as their ultimate goal the restoration of a functional glans and penis with a slit-like meatus and adequate glanular approximation. (67) Urethral plate (UP) is a key anatomical component of the hypospadias phenotype that is recently being more utilized for the ultimate surgical repair and thus knowing its impact on the post hypospadias repair outcomes and its precise appraisal and quantification would be important for objective surgical decision making and prognostication that can further guide parental counselling. Variability in UP features is one of the factors that influences the selection of corrective techniques for hypospadias. However, the UP was not objectively assessed, which has led to debates and the use of ambiguous words to define its qualities in the literature. For this purpose, we conducted a three-phase exploration of this goal.

5.2 Methods and key results

Firstly, a systematic review of the previously stated methodologies used to assess and evaluate UP quality was conducted, highlighting the advantages and disadvantages of each system, and stressing its potential influence on a variety of postoperative outcomes. It was concluded that the postoperative outcomes of hypospadias correction may be estimated in part by judging the quality of the UP, however this assessment is highly subjective. It was also concluded that detailed characterization of the topographical features of penile anatomy would optimize penile reconstruction towards better results. Since there have been so few research done on the glans penis, it has been left to the discretion and judgment of the operating surgeon to determine what constitutes a "normal" shape in terms of meatus length and the extent of the glanular closure line.

The purpose of the second phase was to lay the underpinning for future hypospadias reconstructive surgeries by developing a "nomogram" for the meatal and glanular closure dimensions. (**Figure 7**) 94 male children participated in the study during ritual circumcision (mean age 5.9 years, range 0.6–13). The length of the meatal aperture was 5.3 (±1 mm), while the length of the ventral glans closure was 4.8 (±1 mm). In addition, there was a correlation between the meatal opening size and the glans closure line (r = 0.36, confidence range 0.14–0.54, P .001). It was concluded from this study that the location and size of the meatus opening are consistent in normal male children, and the ventral glans closure is equivalent to or slightly shorter than the meatal length. (**Figure 7**)

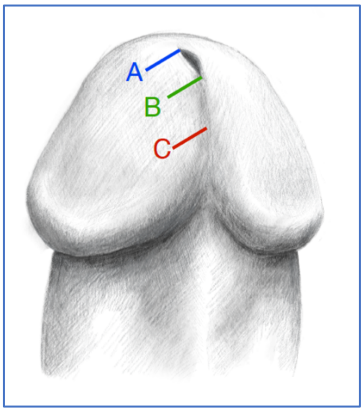

Figure 7. An illustration that shows the several places at which measurements were taken. The proximal extent of the meatal opening and the distal limit of the glanuar closure line are shown at point (B), while the distal extent of the meatal opening is shown at point (A), and the proximal limit of the glanuar closure line is shown at point (C). The distance from A to B defines the meatal aperture, while the distance from B to C defines the glanular closure line in its vertical orientation.

Following that, the inter and intra observer reliability were examined of a new method to assess the quality of the UP in hypospadias (POST - Plate Objective Scoring Tool) based on features of glans characteristics. (**Figure 8**) The Glans-Urethral Meatus-Shaft (GMS) score was used as a gold standard to assess the validity of the scoring system. When compared with the GMS, the POST score performed better and showed superior inter-observer agreement.

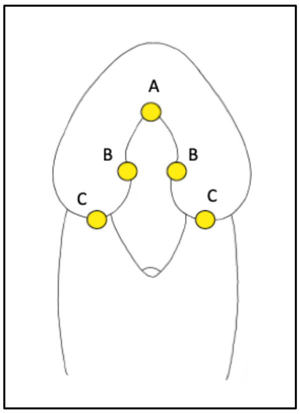

Figure 8. An illustration showing the key anatomical landmarks required to measure the POST score. (A) The distal muco-cutaneous junction of the urethral plate, (B) the glanular knobs, (C) the start of the coronal sulcus. Plate objective scoring tool is equal to AB/BC.

5.3 Conclusions

Based on these studies, it was concluded that the POST score is a useful adjunct to the UP evaluation and may be used as a further metric to gauge success after distal hypospadias repair.

Chapter 6: Investigating the scrotal base distance in hypospadias cohort

This chapter summarizes the results and main conclusions of the study published in the following paper:

- Abbas TO, Ali M. Scrotal base distance: A new key genital measurement in males with hypospadias and cryptorchidism. Curr Urol. 2021 Dec;15(4):214-218. doi: 10.1097/CU9.0000000000000031. Epub 2021 Jun 23. PMID: 35069085; PMCID: PMC8772709. (Appendix; Page 30)

6.1 Introduction

It is well-established that the incidence of hypospadias, undescended testes (UDT), and testicular cancer is on the rise worldwide, with wide geographical variability that may be impacted by their pathogenesis background. (68) Although the causes of this group of diseases have yet to be identified, they fall under the overarching framework "testicular dysgenesis syndrome" (TDS). The abnormal testicular development in this patient group may be due to fetal endocrine disruption (ED) caused by exposure to various environmental chemicals. (69) Multiple animal studies supported this explanation. (70,71)

Anogenital distance (AGD) is sexually dimorphic in a number of mammals, with males having a longer AGD than females. AGD is also regarded as an objective biomarker of fetal endocrine disrupters in humans (72) and a reliable indicator of fetal exposure to androgens and anti-androgens. Consequently, testicular dysfunction in postpubescent males due to fetal exposure to ED is reflected by a shortened AGD. (73,74) It was also demonstrated that patients with hypospadias and cryptorchidism had a shorter AGD (75) because of a suboptimal exposure to endocrine stimuli during a critical fetal period can result in abnormal development of both the internal and external genitalia, with an associated risk of developing hypospadias, UDT, and abnormal sperm production. (76) Assessing AGD has some limitations, including an ill-defined landmark for the posterior end (mid-anus, anterior anal verge), which makes its measurement problematic, especially in children younger than 5 years old who have a large fat pad in the perineum. A new parameter, "Scrotal Base Distance" (SBD), was introduced and examined as a mean to reflect on the development of external genitalia in patients with undescended testis or hypospadias in comparison to TDS. This measurement was chosen because it reflects on size of the scrotum and the testis within it thus a useful determinant of in utero genital development.

6.2 Methods

For this purpose, the SBD of patients with hypospadias or cryptorchidism were compared with a cohort of full-term healthy boys undergoing standard ritual circumcision using age-specific standard deviation scores.

6.3 Key results

In patients with hypospadias or UDT, the mean SBD, AGD, and penile length measurements were shorter than in the control group. This indicates a relationship between this physical biomarker and the severity of the endocrine disruption.

6.4 Conclusions

This study showed that the current infant anthropometric tools (such as AGD) are less consistent than SBD. AGD measurements rely on poorly defined soft tissue anatomical landmarks. However, SBD can be easily measured on easily accessible anatomical landmarks, allowing for the possibility of high reproducibility.

Chapter 7: Employing ultrasonography for the preoperative assessment of hypospadias

This chapter summarizes the results and main conclusions of the study published in the following paper:

- Abbas TO. Ultrasonographic Evaluation of the Hypospadiac Penis in Children. Front Pediatr. 2022 Jul 6; 10:932201. doi: 10.3389/fped.2022.932201. PMID: 35874590; PMCID: PMC9299257. (Appendix; Page 35)

7.1 Introduction

Describing the anatomy of hypospadias is crucial for comprehending and managing this disease and will enable surgeons to select the most effective repair treatments, resulting in greater functional and cosmetic outcomes. (77) This study aimed to discover the precise conformation of the corpus spongiosum in hypospadias instances.

7.2 Methods

The spatial relationships between the corpora cavernosa, corpora spongiosa, hypospadias meatus, and glans penis were determined using penile ultrasonography (PUG). Furthermore, the extent of urethral hypoplasia, and urethral defect were then compared with PUG findings. (**Figure 9).** Prior to surgical correction, 25 children with hypospadias were evaluated using PUG, under general anesthesia in the sagittal and transverse planes.

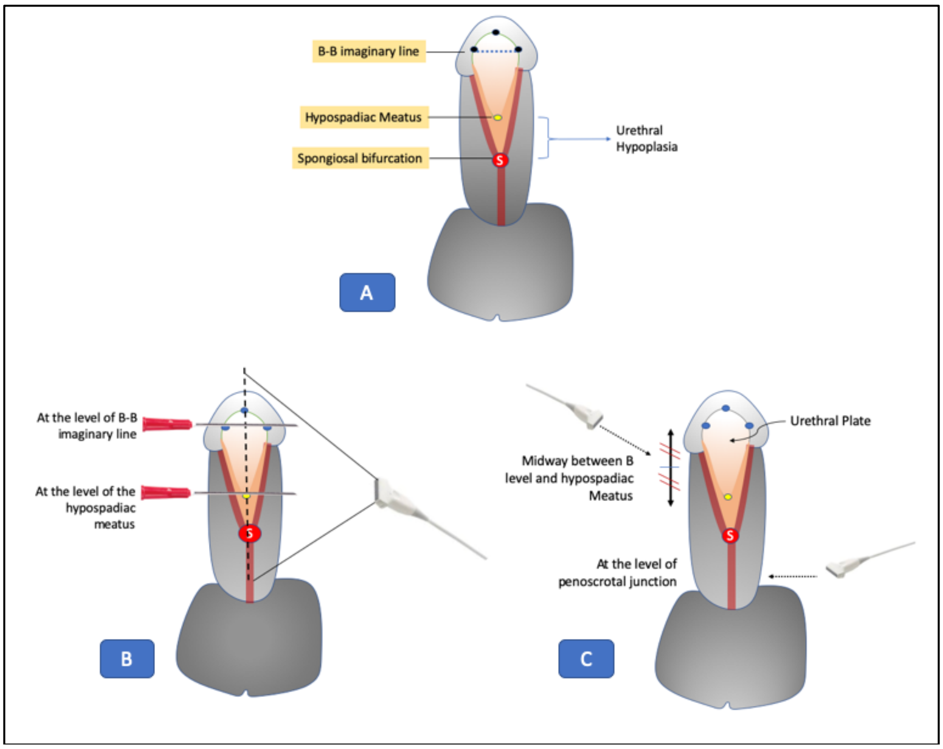

Figure 9. (A–C) Illustrations demonstrating main anatomical points. (A) Diagram displaying the diverse anatomical landmarks used to define urethral hypoplasia. (B) Longitudinal sagittal PUG imaging over the midline. (C) Two transverse PUG images are captured.

7.3 Key results

The amount of urethral hypoplasia indicated by PUG was comparable to intraoperatively acquired values.

7.4 Conclusions

It was concluded that in children with hypospadias, PUG is a practical and reliable method for assessing the penile configuration. Since the PUG accurately measured the distal extent of the spongiosal component of the urethral plate, it may be used to optimize surgical planning and evaluation of current repair techniques.

Chapter 8: Employing artificial intelligence for the automated quantification of the urethral plate quality and the degree of penile curvature

This chapter summarizes the results and main conclusions of the study published in the following paper:

- Abbas TO, AbdelMoniem M, Chowdhury MEH. Automated quantification of penile curvature using artificial intelligence. Front Artif Intell. 2022 Aug 30;5:954497. doi: 10.3389/frai.2022.954497. PMID: 36111321; PMCID: PMC9468331. (Appendix; Page 41)

8.1 Introduction

Artificial intelligence is a cutting-edge technology capable of autonomously segmenting, classifying, and registering medical images. Medical image analysis increasingly employs computer-aided diagnosis based on deep learning. (78) Although the implementation of artificial intelligence and machine learning is currently in its early stages, hypospapdias is one of the potentially most benefitable fields. We intended to use the cutting-edge technology of artificial intelligence to aid in the management of two crucially essential clinical issues in hypospadias care namely, penile curvature (PC) and urethral plate (UP). PC refers to an abnormally bowed penile shaft that can arise either in congenital or acquired male external genital diseases. The incidence of the most prevalent congenital associated pathology, hypospadias, is approximately 1:250 male live births, and between one-fourth and one-third have concomitant significant PC. (3,79,80) The degree of PC can greatly affect the intrinsic severity of hypospadias (81,82) and has a massive effect on the surgical decision-making for the eventual surgical operation. In addition, PC is linked to increased adult dissatisfaction and sexual issues if it is left untreated or poorly managed (83–87) Secondly, it was demonstrated in our earlier systematic study, (77) it is readily apparent that the quality of the urethral plate (UP) could influence the selection process between such procedures and alter post-operative outcomes. However, past approaches for evaluating UP quality are highly subjective and have a low generalizability index. The plate objective scoring tool (POST) (Please see Chapter 5) **(figure 8)** was developed as a reliable and accurate method for quantifying the UP quality and as a guide for selecting particular surgical procedures based on this. (82) Individual evaluations of urethral plate quality and POST are, however, still susceptible to subjectivity particularly when captured from 2D images. Despite attempts at standardization, this variation in assessment may make it difficult to compare the outcomes of different centers and surgeons. The purpose of this research was to optimize the utilization of the POST score by applying artificial intelligence and machine learning to streamline its operation and provide a user-friendly platform that makes measuring POST much more consistent and reproducible.

8.2 Methods

To develop and evaluate the suggested framework of PC angle assessment, nine 3D printed penile models with varying curvature angles (range from 18° to 90°) were used to generate a 900-image dataset comprising diverse camera positioning, inclination angles, and background/lighting circumstances. The proposed approach includes three phases: automatic penile region localization, shaft segmentation, and curvature angle estimation. Smartphone-taken photos of penile models were used to train and evaluate a Yolov5 model to automatically crop the penile portion from the obtained image. Then, an Unet-based segmentation model was trained, verified, and evaluated to segment the penile shaft, and a novel Hough-Transform-based angle estimation technique was employed to estimate the degree of PC.

8.3 Key results

A fully automatic application was developed, requires no surgical intervention, and relies solely on the execution of a novel algorithm for segmenting the penile shaft and calculating PC angles automatically. It has not previously been possible to measure PC using machine learning; consequently, we sought to construct and evaluate a comprehensive AI system for this purpose.

On the other hand, the three essential landmarks of A, B, and C of the POST were annotated in a dataset of 361 images of prepubescent boys having primary hypospadias repair. **(figure 8)** This dataset was then used to construct and verify a deep learning-based landmark detection model. The projected coordinates were then utilized to calculate the AB/BC ratio for an accurate evaluation of the UP's quality. Using a Normalized Mean Error (NME) of 0.1651, the suggested model performed accurately in locating the landmarks. In addition, a Mean Squared Error (MSE) of 0.0015 was attained while estimating the landmarks' coordinates.

8.4 Conclusions

This AI program employing machine learning demonstrates robustness and a high level of precision in evaluating the quality of UP using POST. Further evaluation of an international image-based database is continuing, and the findings of the validation are awaited; these could be used to feed deep learning algorithms and possibly improve surgical outcome predictions. Using the results of both these specific studies regarding the standardization of evaluation tools for the classification of hypospadias severity, penile curvature degree, and urethral plate quality ***(i.e. chapters 4-8),*** the following algorithm was developed to facilitate objective decision making in the management of hypospadias. (**Figure 10**).

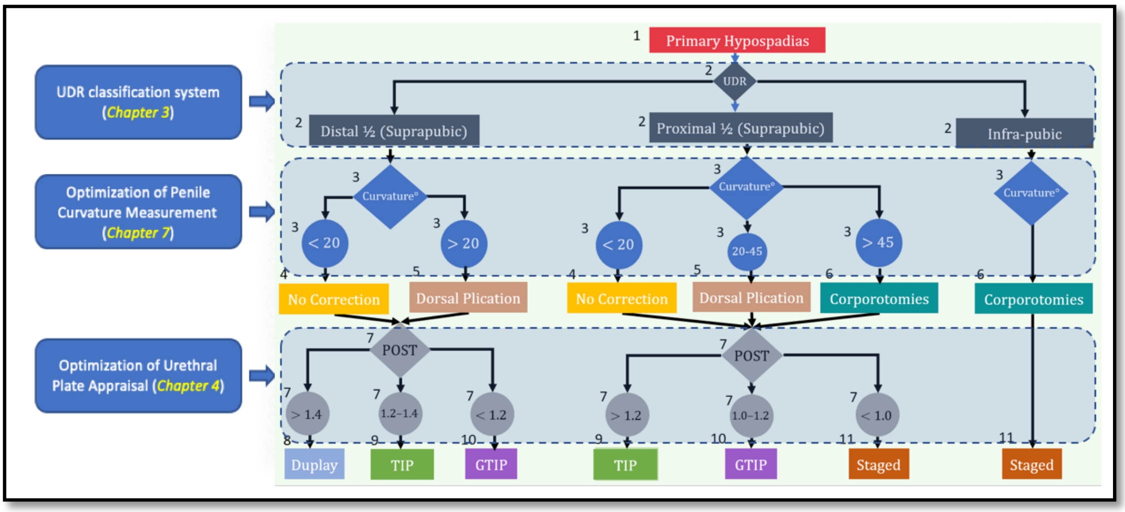

Figure 10. A new objective algorithm for the management of hypospadias based on the outcomes of the three key clinical studies to optimize the hypospadias evaluation tools.

Chapter 9: Creation of a biomimetic scaffold with angiogenesis potential

This chapter summarizes the results and main conclusions of a published review and a manuscript in preparation:

- Abbas TO, Mahdi E, Hasan A, AlAnsari A, Pennisi CP. Current Status of Tissue Engineering in the Management of Severe Hypospadias. Front Pediatr. 2017; 5: 283. (Appendix; Page 52)
- Abbas TO, Parangusan H, Yalcin, H, Hassan M, Zakrif L, Zandi N, Pennisi, CP. Trilayer chitosan/PLA composite scaffold for urethral reconstruction: evaluation of mechanical, biological, and angiogenic properties (*manuscript in preparation*) (Appendix; Page 60)

9.1 Introduction

A crucial objective of tissue engineering is to produce grafts that closely match the natural architecture that is being replaced. The typical human urethra has a tubular shape with three different layers. The majority of tissue engineering studies to date have constructed cell-seeded monolayers or rarely bilayer scaffolds that reconstitute only the epithelial and muscle layers, thereby overlooking the submucosa and the critical role of the surrounding corpus spongiosum in providing mechanical support and blood supply (24,44,88,89). The corpus spongiosum is absent in hypospadias (87,90); hence, failure to restore this tissue may result in long-term failure of urethral reconstruction (91). Therefore, it is expected that the combination of designed spongiosum tissue and urethral mucosa would result in more effective surgical repair and improved functional outcomes. Major obstacles to urethral regeneration with standard tissue-engineered grafts are inadequate angiogenesis and inadequate epithelialization. An ideal tissue-engineered urethra should promote epithelialization and urethral regeneration in vivo with appropriate angiogenesis.

Different materials that can substitute autologous grafts in reconstructing the urinary system would significantly reduce surgical problems. However, acellular materials have proven insufficient for this purpose, and the production of cell-seeded matrices is technically complex and time-consuming. In contrast, tissue-engineered scaffolds that blend synthetic and natural biodegradable materials have demonstrated significant promise in regenerative medicine.

9.2 Methods

Using a sequential electrospinning technique, poly-L-lactic acid (PLA) and chitosan (Chi) were used to create microporous tri-layer scaffolds (CPC). Morphology, water absorption, mechanical properties, and degrading parameters were evaluated and compared to monolayer PLA scaffolds and natural collagen-based scaffolds (Alloderm, ALD). The biological properties of the scaffolds were evaluated in vitro by cytotoxicity, migration, and proliferation assays. A chick embryo chorioallantoic membrane (CAM) experiment was performed to assess the angiogenic properties of the scaffolds.

9.3 Key results

The average thickness of the CPC scaffolds was 1.10±0.19 mm. On the other hand, the PLA scaffolds had an average thickness of 0.60±0.12 mm, while the ALD scaffolds had a thickness of 2.30±0.29 mm. CPC scaffolds showed a linear stress-strain characteristic until failure at high load. In contrast, the PLA scaffolds exhibited a less stiff behavior, while the ALD scaffolds exhibited the largest deformation at low stress. contact angle measurements indicated that CPC was significantly more hydrophilic compared to the other scaffold types. CPC scaffolds were able to effectively support SMC growth on both sides of the scaffolds, in contrast to PLA scaffolds where cells appeared to detach from one of the sides. Using the CAM assay, the CPC scaffolds caused a significant increase in blood vessel size and number of vessel branches compared with the control. The increase in vessel diameter was comparable to that induced by the ALD scaffolds. In contrast, PLA scaffolds had a significantly lower number of vessel branches and no significant effect on vessel diameter.

9.4 Conclusions

CPC scaffolds possess properties that make them an attractive candidate for surgical repair of hypospadias and long-segment urethral strictures. The nano/microfiber structure of the chitosan layers increases scaffold stiffness, making it easier to handle and process, and may provide sufficient mechanical properties to maintain an open urethra in the long term. CPC scaffolds also promote fibroblast migration and angiogenesis, both of which are required for effective tissue healing. Overall, CPC scaffolds appear to be a viable alternative to autologous tissue reconstruction for the repair of hypospadias and complex long-segment urethral strictures. In vivo testing in a suitable animal model should be conducted in the future to determine whether these trilayered scaffolds can support host cell ingrowth while also functioning as an efficient graft for urethral repair.

Chapter 10: Quality assessment of reporting of preclinical animal experiments in hypospadias repair and urethral tissue engineering

This chapter summarizes the results and main conclusions of the study published in the following papers:

- Abbas TO, Elawad A, Kareem A, Pullattayil S AK, Ali M, Alnaimi A. Preclinical Experiments for Hypospadias Surgery: Systematic Review and Quality Assessment. Front Pediatr. 2021 Aug 9; 9:718647. doi: 10.3389/fped.2021.718647. PMID: 34458213; PMCID: PMC8386350. (Appendix; Page 91)
- Abbas TO, Elawad A, Pullattayil S AK, Pennisi CP. Quality of Reporting in Preclinical Urethral Tissue Engineering Studies: A Systematic Review to Assess Adherence to the ARRIVE Guidelines. Animals (Basel). 2021 Aug 21;11(8):2456. doi: 10.3390/ani11082456. PMID: 34438913; PMCID: PMC8388767 (Appendix; Page 103)
- Book Chapter: Tariq O. Abbas, Petra De Graaf, Cristian Pablo Pennisi. The rabbit model in preclinical hypospadias research: strengths and limitations. (Springer) Hypospadiology; Current Challenges and Future Perspectives. (2023) (Appendix; Page 118)

10.1 Introduction

There is widespread agreement that preclinical experiments are essential for making substantial progress toward the creation of novel therapeutics. Although other models with greater translational significance may be obtainable, the selection of an animal model is ultimately dependent on the researchers' knowledge, ethics, logistics, and budget (52). Even though many animal models have been utilized (44,53), in the domain of urethral repair, the rabbit model has been the most common due to the ease with which the urethra of the animal can be accessed. Favorable conclusions drawn utilizing cell-laden matrices in preclinical research could not be translated to clinical studies, according to a recent meta-analysis of 63 preclinical and 13 human studies of tissue engineering for urethral reconstruction (44). Poor quality animal experiments are one of the main reasons that hinder the translation of findings (92). There are many other explanations why the data found in preclinical studies is not replicated in the subsequent clinical trials. In 2009, the NC3Rs investigated the extent to which 271 previously published animal studies had followed accepted standards for reporting, experimental design, and statistical analysis. Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines were published in 2010 (93,94) in response to the survey's revelations of flaws in experimental design, statistical analysis, and reporting. The 20-item checklist is designed to ensure that all the necessary information for a preclinical scientific publication is included. The NC3Rs recently put together an international working group made up of journal editors, researchers, and statisticians from a wide range of fields. This group is constantly revising the checklist items in light of major advances in methodological approaches (95). While several reviews have summarized and discussed the pros and cons of the various biomaterials used in clinical and preclinical experiments on urethral tissue engineering (24,44,53,54), these articles have primarily focused on the results of the studies. There is only a single publication that has evaluated the quality of the reported preclinical experiments, and even that only used a small subset of quality parameters (44), like details about the animals used, the methods used, and the results obtained. Since the ARRIVE guidelines are a standardized and up-to-date document that permits a comprehensive revision of preclinical research, this checklist was used to evaluate the quality of reporting in studies of urethral repair for hypospadias reconstructive procedures and using tissue engineering approaches. As rabbits are the most used animal model for preclinical studies in urethral reconstruction, they are the primary focus of the quality evaluation. Data is extracted into an ARRIVE-inspired, standardized framework.

10.2 Methods

Using the inclusion and exclusion criteria, 30 studies were selected for full-text screening that investigated surgical methods for hypospadias in animals; 21 of these met the criteria for a quality evaluation. In September of 2020, a search was performed in the PubMed MEDLINE database and the OVID SP EMBASE database. Terms like "urethra," "urethroplasty," "urethral reconstruction," "urethral graft," "animal experiments," "reconstructive surgery," and "tissue engineering" were proposed as search terms. Full-text versions of all articles that qualified were analysed. Three independent reviewers extracted the data into a standardised data framework based on the ARRIVE principles (96). Items were scored as "yes" if they were mentioned in the study, "no" if they were not, and "n/a" if they were not relevant. We utilised the SYRCLE (97) and its 10 signaling questions to assess the potential for bias in the research. The Mann-Kendall test was used to compare the mean and standard deviation of the articles' scores across publication years. The threshold for significance was determined to be P 0.05.

10.3 Key results

An average of 66% was attained upon completion of the checklist in studies of hypospadias and 53% of tissue engineering urethroplasty studies. The use of a sufficient sample size, the separation of test subjects from controls, and the clarity with which results were presented all received perfect scores. Information about the experimental method, housing and husbandry, rationalization of the number of animals, and reporting of adverse events were the least frequently stated items. No published work provided a predicted sample size. Conversely, 28 articles meeting the criteria for inclusion were drawn from the preclinical literature on urethral tissue engineering. The ARRIVE score could be anywhere from 0 to 100, depending on whether the item in question has been reported. The typical result on a checklist was a 53. The use of animals, the size of the control and experimental groups, and the description of the results of the experiments all received the highest scores. Procedure details, housing and husbandry information, animal count justifications, and reports of adverse events ranked among the least frequently reported aspects of experiments. Surprisingly, only 46% and 54% of studies adequately explained how they adhered to ethical guidelines and received approval for their use of animals in hypospadias and urethral tissue engineering studies respectively. No published work provided a predicted sample size neither in hypospadias nor urethral tissue engineering preclinical studies

10.4 Conclusion

Overall, this research showed that many studies fail to adequately report necessary details, despite some progress in reporting quality over the course of the study. It is strongly advocated the full adoption of the ARRIVE guidelines in animal studies investigating urethral repair, not only to ensure the smooth transfer of knowledge from the laboratory to the clinic but also to guarantee adherence to ethical principles and reduce the number of animals used. To ensure translatability and reproducibility, it is emphasized some of the most critical issues to define when designing preclinical studies of hypospadias are animal age, anatomic location, and injury size.

Chapter 11: General discussion, future perspectives, and conclusions

11.1 General discussion

The ultimate goal of the various surgical procedures to correct hypospadias is to achieve a straight penis and urethral meatus at the tip of the glans by tubularization of suitable tissue. (10) In recent decades, there have been major advances in the treatment of hypospadias. (9) However, numerous elements of the hypospadias phenotype that are considered important anatomic determinants are still subjectively assessed and standardized quantification, classification, and appraisal techniques are lacking. These characteristics include, but are not limited to, hypospadias severity categorization, urethral plate quality, and degree of related penile curvature.

For example, several hypospadias classification systems have been developed to categorize the severity based on the location of the urethral meatus. However, these classification systems fail to accurately reflect the true location of the spongiosal bifurcation, which is the underlying cause of hypospadias and were deemed subjective, with a high inter- and intra-observer variability index. (9) The proposed hypospadias classification system based on the urethral defect ratio (UDR) (98) has been shown to be highly reproducible and significantly linked with other anatomic factors such as the urethral plate quality and degree of penile curvature. Such a comprehensive classification system could improve consistent communication between clinicians and scientists. However, the UDR classification system cannot be accurately identified by visual inspection in the clinic and would ideally be defined following penile skin degloving or with the assistance of an imaging tool such as ultrasonography. (99)

Similarly, the urethral plate (UP) is an important anatomical component of the hypospadias phenotype that is increasingly being used for the final surgical repair; therefore, understanding its impact on post-hypospadias repair outcomes and its precise quality quantification would be essential for objective surgical decision-making and prognosis, which can further guide parental counseling. Although UP quality is an independent factor in hypospadias treatment postoperative outcomes (77), current approaches for evaluating UP quality are highly subjective and have a low generalizability index. On the basis of observational study on normal children undergoing ritual circumcision to analyze the normal topographical characteristics of the glans penis. (100) Then, the plate objective scoring tool (POST) was devised as an objective method for assessing the urethral plate quality. (82) It was further shown that the POST is an objective, dependable, and consistent method for assessing UP quality and aiding in surgical decision-making. In addition, artificial intelligence (AI) was utilized to improve the precision and accuracy of measuring the POST and built a *platform* (https://hypospadias-ai.netlify.app) that is accessible to the public. In a similar manner, we used AI to objectively and automatically quantify the degree of penile curvature, which is a crucial anatomical feature but is inconsistently and inadequately measured. (101) Due to the fact that this study was conducted using 3D-printed penile models, its applicability to the intraoperative setting may be limited, and additional research is ongoing. Collectively, standardization of the measurements of severity of hypospadias, urethral plate quality and degree of penile curvature had allowed us to implement and functionalize a step-by-step decision-making algorithm for the management of primary hypospadias cases. **(Figure 10).**

In addition, a new metric, "Scrotal Base Distance" (SBD), was created and tested to reflect on the development of external genitalia in patients with undescended testis or hypospadias versus testicular dysgenesis syndrome. (102) The mean SBD, anogenital distance, and penile length measurements were shorter in patients with hypospadias or undescended testis than in the control group (boys for standard ritual circumcision). This suggests a link between this physical biomarker and the degree of endocrine disturbance. SBD is simple to quantify on easily accessible anatomical landmarks, allowing it to be repeated several times. To further explore the anatomical configuration of the hypospadias anomaly, penile ultrasonography, was used to study the spatial relationships between the corpora cavernosa, corpora spongiosa, hypospadias meatus, and glans penis were determined. This would provide patients with the disease with improved functional and cosmetic outcomes. (77)

A nanofibrous scaffold of Chitosan/PLA/Chitosan was produced, comprising a structure like the three-layer structure of the urethral wall. The incorporation of nanofibrous components into the tri-layer material resulted in scaffolds that were easier to bend, shape, and manipulate without tangling or sticking. The CPC scaffold has high suture retention, which is important for successful graft implantation, and its increased wettability due to the chitosan layers allows for efficient cellular infiltration and growth. The tri-layer membrane promoted effective cell growth in vitro and angiogenesis and may provide a unique tissue-engineered replacement for the urethra in vivo. Chitosan-based materials have previously also been shown to activate polymorphonuclear cells and fibroblasts, influence cytokine production, promote giant cell migration, and stimulate type IV collagen synthesis, which collectively support wound healing. (103) Next step is to investigate the anatomical and functional outcomes following the implantation of these scaffolds in an animal model.

At the same time, there is a broad consensus that preclinical studies are necessary to make substantial progress in the development of new treatments. Although numerous animal models have been used (44,53), the rabbit model is the most commonly used model in the field of urethral repair because of the easy accessibility of the urethra. The use of an adequate sample size, the separation of test subjects from controls, and the presentation of results were all rated as flawless. (104,105) However, the information regarding the experimental procedure, animal housing and husbandry, the rationalization of the number of animals used, and the reporting of adverse events was the least frequently mentioned. These findings may explain why animal experiments are not optimally applicable to human investigations. (92) In 2009, the NC3Rs investigated the extent to which 271 previously published animal studies had followed accepted standards for reporting, experimental design, and statistical analysis. Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines were published in 2010 (93,94) in response to the survey's revelations of flaws in experimental design, statistical analysis, and reporting. The 20-item checklist is aimed to ensure that a preclinical scientific publication contains all required material. Recently, the NC3Rs established an international working group comprised of journal editors, academics, and statisticians from a variety of disciplines. Since rabbits are the most often utilized animal model for preclinical investigations in urethral repair, they are the primary focus of the quality assessment. It is encouraged the complete adoption of the ARRIVE standards in animal research examining urethral repair, not only to facilitate the easy transfer of knowledge from the laboratory to the clinic, but also to assure adherence to ethical principles and limit the number of animals utilized. Journal editorial committees have advocated strict standards as a means of enforcing the aforementioned reporting quality and behavior change (106). To ensure translatability and reproducibility, when designing preclinical studies of hypospadias, animal age, anatomic location, and injury size are some of the most important factors to characterize.

11.2 Limitations

There are a number of limitations in the studies, any or all of which could be addressed and overcome in future research. The absence of data on the relationship between meatal length and closure line and other biometric measurements of the penis, such as glans size, and the small sample size of the study conducted to investigate the biometrics of the size of the urethral meatus and glanular fusion line are two significant limitations.

A limitation of the ultrasonographic investigation, on the other hand, is that it involved only one measuring surgeon, so we were unable to analyze inter-observer variability. In addition, there was no formal blinding system implemented, despite the fact that the sequential evaluation of eyeballing, ultrasonographic, and surgical data may have been suitable for such a feasibility study. Therefore, future study must assess the interobserver variability and reproducibility to validate these findings.

As with any review, one of the review's weaknesses is the potential subjectivity of the assessment undertaken by the different evaluators of the systematic review for the reporting quality of preclinical urethral investigations. However, the substantial agreement across observers indicates that the raters implemented these rules in a largely consistent manner. The small number of included studies also made it difficult to draw broad conclusions regarding the factors that influenced study reporting. It was decided not to construct a summary score for each study using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) because doing so would require assigning "weights" to certain categories in the tool, which would be subjective. Due to the presence of several subitems, our operationalization table has the potential disadvantage of counting incomplete reports as "Yes." Because of this, we have included a sub-item analysis to establish how well each sub-item is followed, as opposed to just how well the item as a whole is followed.

11.3 Future Perspectives

11.3.1 Standardization of the assessment of hypospadias anomaly

Although the studies conducted in this work have shown important progress in optimizing the tools that can be used to standardize the evaluation and treatment of hypospadias, the practical application of such tools in a large cohort of patients' needs to be explored. Creating randomized studies based on the various UDR categories, POST scores, and curvature degrees could objectively validate these instruments. At the same time, the artificial intelligence-assisted study of pooled databases integrating genetic, phenotypic, and surgical care characteristics could bring more clarity on the development and management of hypospadias abnormality.

11.3.2 Preclinical urethral studies

The literature review on the status of tissue engineering in hypospadias revealed that longer follow-up periods and more realistic animal models are necessary for future research in this field to assist bridge the gap between animal models and clinical investigations. We believe that biodegradability is crucial for the success of tissue-engineered urethral regeneration in children, which may coincide with the increase in penile size during puberty. The incorporation of biologically active agents into acellular natural or synthetic matrices represents the emerging field of "smart biomaterials," which may one day capitalize on recent advances in understanding the physical and chemical factors responsible for urothelial and smooth muscle cell proliferation, migration, differentiation, and function. Through interactions not just between the urothelium and stroma, but also between tissue-engineered grafts and the host tissue environment, tissue remodeling can be improved. "dynamic mutuality"(107) is the term used to describe this idea. We can better comprehend the biological complexity and physicochemical characteristics of native tissue by actively cooperating across disciplines, such as polymer chemists, biomaterials scientists, tissue engineers, and doctors.

11.3.3 Urethral tissue engineering

Preclinical validation of the suitability of the fabricated trilayer scaffolds in a suitable animal model is imperative. Careful attention to the intricacies of research design and reporting in accordance with the PREPARE and ARRIVE recommendations would go a long way toward addressing most of the deficiencies in existing reports. In addition, the development and use of a more "realistic" pathologic animal model involving either hormonally induced urethral defect or chronic urethral lesions could lead to more generalizable results.

11.4 Conclusions

Two objective scoring tools have been developed and validated, including the urethral defect ratio for classifying the degree of hypospadias and the plate objective scoring tool for evaluating the quality of the urethral plate. Both clinical standardization tools can help to enforce objective communication between surgeons and researchers, as well as standardize variable categorization when conducting prospective studies. At the same time, ultrasonography and cutting-edge artificial intelligence were used to characterize several hypospadias anatomical features and demonstrated to reduce inter- and intra-observer variability and increase reliability. This can also help in non-invasively evaluating different anatomical variables and proposing new etiological hypotheses and outcome predictors based on large patient data sets using machine learning. On the other hand, acellular tri-layer scaffolds with enhanced regenerative properties were developed for urethral applications, adding an option for off-the-shelf urethral graft resources after further optimization and validation. Simultaneously, in accordance with the ARRIVE standards for animal research, a review of the reporting quality in preclinical studies using tissue engineering approaches for urethral repair revealed significant reporting gaps. The adoption of new regulations and policies to compel the use of such reporting tools, particularly those tailored for hypospadias potential, has the potential to improve clinical findings' translatability from animal experiments to human studies.

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