



LATE DEVELOPMENT OF HUMAN MALE URETHRA AND PREPUCE: A HISTOLOGICAL & HISTOCHEMICAL STUDY

Authors: Ramesh Babu¹, Sathyamurthy Arunaa¹, Kalaivanan Chitra², Leena Dennis Joseph², Ahmed Hadidi³

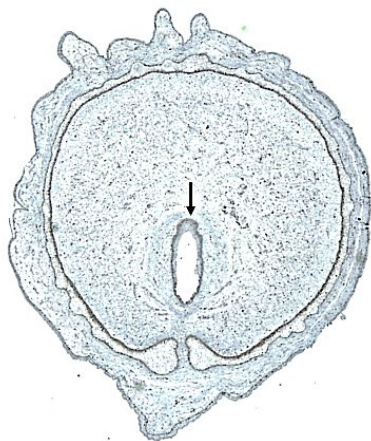
Affiliation: Dept of Pediatric Urology¹ & Pathology² Sri Ramachandra Institute of Higher Education & Research, Chennai, India; Department of Pediatric Surgery, Emma Klinik GmbH, D-63500 Seligenstadt, Germany³

Purpose: Previous studies examined early development of human male urethra upto 14 gestational week. We examined and analysed mid-trimester fetuses to explain the late stages of urethral development and prepuce.

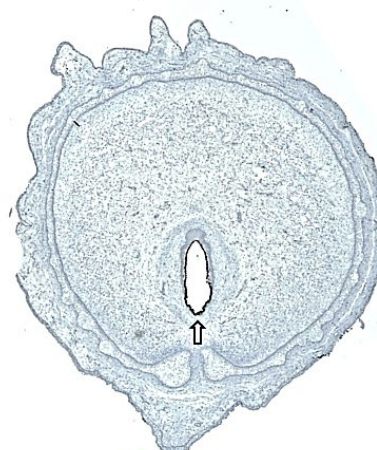
Materials & Methods: Fifteen human male fetuses ranging from 14 to 25 gestational weeks were examined after obtaining ethics committee approval. Serial histological sections were stained using haematoxylin and eosin as well as immuno-histochemical stains (CK 7 and MIB Ki67).

Results: 1) During the 14-17 weeks the glanular urethral plate was mainly solid, filled with squamous epithelial cells. Vacuolation and canalization have started causing the protrusion of epithelial tag outside the glans. The proximal urethra seemed developed, horizontal in orientation and surrounded by well organized corpus spongiosum. 2) At 17-20 weeks: The orientation of the urethra changed from horizontal at penile level to star-shaped at fossa navicularis and vertical at glans. Proliferating epithelial cells in the roof stained positive for MIB Ki67, while embryologically different urothelial cells in the floor stained positive for CK7. Prepuce formed floor of the glanular canal through the frenulum. The spongiosa did not extend into the glans but stopped at navicular fossa. 3) At 20-24 weeks: The glanular urethra continued vacuolation and fusion though the frenulum. Development of the frenulum and prepuce was complete by 24-weeks.

Conclusions: The development of proximal urethra was complete by 14-weeks gestation. However glanular urethra/ prepuce development continued till 24-week gestation. This may explain why distal hypospadias is more common. The present study may explain megameatus intact prepuce where lack of the frenulum results in a wide glanular canal without floor. Further studies on human male embryos are needed to support these findings



Proliferating cells (stratified squamous) seen in dorsal aspect of glanular urethra & beneath prepuce both stain positive for MIB Ki67



Non-proliferating (columnar cells) seen in ventral aspect of urethra stain positive for cytokeratin CK7



The effect of preoperative hormonal stimulation on the urethral plate; A histologic and histochemical study

Authors: Michael Sennert¹, Christina Perske², Mohamed Fawzy¹, Johannes Wirmer¹, Ahmed T Hadidi¹

Affiliation: ¹Hypospadias Center, Department of Pediatric Surgery, SANA-Klinikum Offenbach, Germany; michaelseennert@yahoo.de

²Institute for Pathology, University Medical Center Goettingen, Germany.

Aim of the work

Preoperative hormone stimulation (PHS) is used to increase the glans size and may improve the cosmetic appearance after hypospadias surgery. The exact effect of PHS on different penile tissues remains unclear and controversial. Previous studies showed that PHS increased vessel density in the foreskin. However, the effect of PHS on the urethral has never been studied before. In this study we examine the PHS effects on the urethral plate.

Materials and Methods

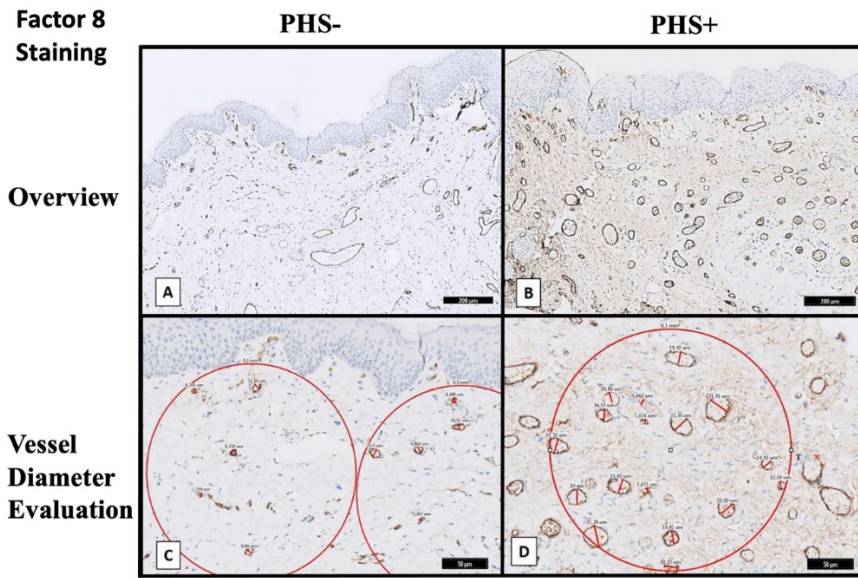
Specimens of the urethral plate and the underlying tissue were excised to correct severe chordee in 16 children with proximal and perineal hypospadias with severe chordee in 2 groups. Group A consisted of 8 children received PHS prior to surgery. Group B included 8 children with proximal and perineal hypospadias with severe chordee did not receive PHS and served as a control group. Specimens were examined blindly using hematoxylin-eosin and elastic van-Gieson stain as well as factor 8 and SMA antibodies.

Results

The median age of Group A was 13 months. The median age of Group B was 17 months ($p=0,03$). The median vessel-density in group A (82 vessels per mm^2) was significantly higher than in Group B (65 per mm^2), ($p<0.05$). The median vessel diameter was also significantly higher in Group A ($13\mu\text{m}$) than in Group B ($11\mu\text{m}$), ($p<0.05$). There was no significant change in dartos fascia layer thickness or androgen receptor expression. There was no visual change in the organization of elastic fibers.

Discussion/Conclusion

This study is the first study to document the effect of PHS on the urethral plate in hypospadias with severe chordee. PHS significantly increased the median vessel-density as well as median vessel diameter as compared to a matching control group.



Exemplary figure showing factor 8 staining



Buried penis; in pursue of its etiology

Authors: Mohamed Fawzy¹, Christina Perske², Michael Sennert¹, Johannes Wirmer¹, Ahmed T. Hadidi¹

¹Hypospadias Center, Department of Pediatric Surgery, SANA-Klinikum Offenbach, Germany

²Institute for Pathology, University Medical Center Goettingen, Germany.

Summary

Aim of the study: To investigate the histological and histochemical features of dartos fascia in buried penis (BP) as compared to dartos fascia in hypospadias and normal children.

Materials and methods: The study included 40 children, operated on in our centre between January 2023 and December 2023. Patients were divided into 3 groups; Group A: 13 patients with BP, Group B:14 patients with Hypospadias, and Group C with13 patients who were referred for circumcision (control group). All specimens were blindly examined by the same pathologist. The 3 groups were assessed for different histological criteria.

Results:

In group A (BP), there were statistically significant dominance of thick collagen fibres (thick fibres) $p<0.001$, thick smooth muscle fibres ($P<0.001$) and the nerve fibres were thick and convoluted ($p=0.004$) as compared to Hypospadias and control groups.

Although Vater-Pacini tactile bodies were more dominant in the buried penis group, there was no statistically significant difference compared to the other 2 groups.

The hypospadias group had significant predominance of chaotic disorganised nonparallel fibres $p=0.003$, intermediate collagen fibres ($p<0.001$) and long thin and short thin fibres ($p<0.001$).

Discussion:

The etiology and histopathology of buried penis and hypospadias remains unclear. Very few studies in the literature studied the histological differences in these conditions. In this study, we examined the histological and histochemical differences in the fascia in children with buried penis, hypospadias and normal children undergoing circumcision for non-medical reasons.

The limitation of this study is the relatively small sample size, and the scarce data available in the literature.

Conclusion:

The fascia In BP is characterized by abnormally thick collagen fibres and thick smooth muscle fibres. This may explain why the penis is drawn inwards in BP and suggests that it is probably recommended to excise this abnormal fascia to reduce recurrence.



Association Between Chronic Hypertension During Pregnancy and Hypospadias Risk: A Systematic Review and Meta-analysis

Authors: Arry Rodjani¹, Irfan Wahyudi¹, Gerhard Reinaldi Situmorang¹, **Tariq Abbas**², Putu Angga Risky Raharja¹

¹ *Department of Urology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jalan Diponegoro No. 71, Jakarta 10430, Indonesia*

² *Urology Division, Sidra Medicine, Doha, Qatar*

Abstract

Introduction:

Literatures have suggested that hypertensive disorders of pregnancy increase the risk of hypospadias, but none have ever focused on maternal chronic hypertension (CH) in hypospadias occurrences. This study would like to develop a systematic review and meta-analysis of present observational studies to assess the association between CH with risk of hypospadias.

Methods:

Literature searches through EMBASE, SCOPUS, Pubmed, and hand-searches according to PRISMA 2020 guideline and MOOSE checklist. Eligible articles were included in the study and assessed for quality utilizing Newcastle-Ottawa Scale (NOS). Extracted data were presented in review table. Pooled analysis was done for unadjusted and adjusted effect size resulting in OR and 95%CI using DerSimonian and Laird model. Heterogeneity was tested using I^2 test, and publication bias was examined using funnel plot.

Results:

Searches yielded 1,187 publications and seven eligible studies were selected. NOS quality score of selected studies were all high, scored from 6-9. There is an elevated risk of hypospadias occurrences in maternal CH both in pooled unadjusted and adjusted OR (OR 1.46 95%CI 1.18-1.79; OR 1.52 95%CI 1.34-1.72 respectively). Heterogeneity was high in unadjusted pooled analysis and low in adjusted, with $I^2 = 82%$ $P < 0.0004$ and $I^2 = 0%$ $P = 0.62.$, respectively. Funnel plots were symmetrical in both analyses indicating no publication bias.

Conclusions:

This meta-analysis indicate that chronic hypertension increases risk of hypospadias. Future studies on biological mechanisms and pathways should be conducted in to elaborate the diseases' pathogenesis.



IDENTIFICATION OF *LHFP* AS A NOVEL GENE FOR FAMILIAL HYPOSPADIAS: INSIGHTS FROM A STRUCTURED BIOBANK IN QATAR AND WHOLE GENOME SEQUENCING ANALYSIS

Authors: Kholoud AlShafei, Luis Saraiva, [Tariq Abbas](#)

Affiliation: Sidra Medicine, Doha, Qatar

Aim:

Hypospadias is a common congenital anomaly in male infants, involves under development of the ventral aspect of the penile shaft. Genetic factors significantly contribute to the etiology of hypospadias. Identifying genetic contributors especially in understudied populations, can enhance our understanding and inform targeted interventions.

Methods:

This study utilized a structured familial biobank to collect and analyze biospecimens from hypospadias cases and their relatives. Whole genome sequencing (WGS) was performed on 27 subjects from seven families, to identify potential genetic causes. Bioinformatics tools including GEMINI was used to assess segregation of single genetic variants (SNVs) with the condition within families.

Results:

Our analysis revealed a rare splice site variant in *LHFP* gene. The variant was detected in a homozygous state in the two affected siblings who were from Sudan, while parents were heterozygous. The variant is a likely pathogenic variant based on ACMG-AMP classification guideline. *LHFP* gene was not previously related to hypospadias, however, *LHFP12* which belongs to the same gene family have showed previously inappropriate female and male distal reproductive tract development in *lhfp12* mutant mice.

Discussion:

The discovery of *LHFP* as a potential gene related to hypospadias underscores the value of structured biobanking and genetic investigations in uncovering pathogenesis of congenital conditions.

Conclusions:

Our findings provide a new avenue to functionally assess the identified variant(s) in relation to disease, and to better understanding disease pathophysiology and to use findings for informing clinical practice and genetic counseling.



DO PREOPERATIVE LEUKOCYTE AND NEUTROPHIL LEVELS HAVE A VALUE IN PREDICTING URETHRAL DEHISCENCE IN HYPOSPADIAS REPAIR?

Authors: Sadık Abidođlu¹, İsmail Demiryorgan², Aytan Ceren Bakır², Özge Kılıç Bayar², Halil Tuđtepe³, Gürsu Kıyan², Ahsen Karagözlü Akgül¹

1 Marmara University, Faculty of Medicine, Department of Pediatric Surgery, Division of Pediatric Urology, Istanbul, TURKEY

2 Marmara University, Faculty of Medicine, Department of Pediatric Surgery, Istanbul, TURKEY

3 Tuđtepe Pediatric Urology Center, Istanbul, TURKEY

INTRODUCTION AND AIM: Different results can be obtained in the same type of hypospadias performed by the same surgeon and applied the same repair technique. This suggests that there are other unmeasurable factors affecting the results. The aim of our study is to contribute to the literature by determining the value of subclinical inflammation in predicting postoperative urethral dehiscence after hypospadias repair.

PATIENTS AND METHOD: We retrospectively analysed the data of 413 patients who underwent hypospadias repair in our clinic between January 2016 and January 2019. Age at surgery, type of hypospadias, repair technique and complications were recorded. Patients with a single-session hypospadias repair performed by two experienced surgeons were included in the study. White blood cell, neutrophil, lymphocyte, platelet and monocyte levels and mean platelet volumes were recorded from the blood test performed one day before the operation. Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and other parameters were analysed to determine their relationship with complications after hypospadias surgery.

RESULTS: The mean age of the patients was 43.5 months. There was no difference between patients with and without separation in terms of mean age, complication ratio of surgeons and follow-up time. Lymphocyte, NLR, and PLR values were statistically higher in patients with dehiscence in neo-urethra ($p=0.025, 0.018, 0.07$, respectively). In the Roc analysis, the area under the curve (AUC) was determined as 79.1% for lymphocyte. The mean follow-up period was 46.7 months (12-102 months, median=46 months).

CONCLUSION: Lymphocyte was statistically significant in predicting urethral dehiscence after hypospadias repair and AUC was 79%. This result may have an impact on the timing of surgery, such as postponing hypospadias repair to the date when the patient's lymphocyte levels are low.



IS THE INCREASING INCIDENCE OF HYPOSPADIAS SIGNALING A GENETIC ETIOLOGY? A PROSPECTIVE CASE-CONTROL STUDY

Authors: Vikesh Agrawal, Bharath S, Himanshu Acharya

Institute: Netaji Subhash Chandra Bose Government Medical College, Jabalpur, India

Background: With rising global incidence rates and unknown etiology of hypospadias, understanding its genetic underpinnings has become increasingly imperative. In our region, where the prevalence of hypospadias is notably high, over 100 cases are managed annually prompted us to identify genetic predisposing factors.

Aim of the Study: This pilot case-control study aimed to explore genetic polymorphisms associated with hypospadias in a cohort of patients.

Materials and Methods: This was a prospective case-control study conducted in a tertiary centre at Jabalpur, Central India. Between Jan 2018 and April 2023, patients admitted with hypospadias were enrolled genetic analysis focused on 20 children with mid-penile hypospadias and a matched control group of 20 healthy children without congenital anomalies was done. Pre-operative abdominal ultrasonography ruled out associated disorders of sexual differentiation. Blood samples from the study group underwent PCR amplification targeting SRD5A2 and CYP17 genes.

Results: RT-PCR analysis revealed that 4 out of 20 hypospadias cases (20%) exhibited amplification for both SRD5A2 and CYP17 genes, while 2 cases (10%) showed amplification for SRD5A2 alone. [Figure 1a and 1b] None of the control group samples showed amplification for these genes.

Discussion: Genetic polymorphisms in SRD5A2 and CYP17 genes were detected in hypospadias cases but not in controls, suggesting a potential role in pathogenesis. The enzymes encoded by these genes are crucial in androgen metabolism and signaling during fetal development, possibly influencing genital morphogenesis. The SRD5A2 gene encodes the enzyme responsible for converting testosterone into its more potent form, dihydrotestosterone (DHT), critical for male genital development. Similarly, CYP17 is involved in steroidogenesis, influencing androgen biosynthesis pathways. Understanding these genetic factors could lead to targeted interventions and personalized management strategies for hypospadias in the future.

Conclusion: These preliminary findings suggest a potential genetic association between hypospadias and polymorphisms in SRD5A2 and CYP17 genes. Further research with larger cohorts is warranted to validate these findings and elucidate the genetic mechanisms underlying hypospadias pathogenesis.

Keywords: Hypospadias, genetic polymorphism, case-control study, SRD5A2, CYP17, PCR

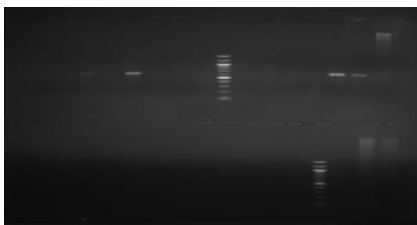


Figure 1a: Amplification of 629 bp product for CYP17 gene exhibited by 4 samples

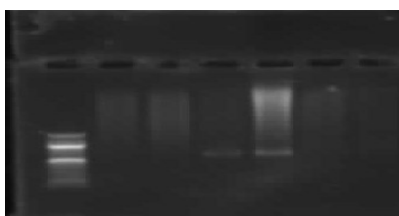


Figure 1b: Amplification of 639 bp fragment for SRD5A2 gene exhibited by 4 samples



Ensuring Cytocompatibility and Biocompatibility of Highly Elastic Nanofibrous Sheets tensile matched to Native Urethra

Authors: Renea Sturm¹, Felix Yiu¹, Zijian Zhong², Ronak Afshari², Ruby Jimenez¹, Rebekah Jung¹, Aljohn Cabuang¹, Nasim Annabi²

¹Department of Urology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA, ²Department of Chemical Engineering, University of California Los Angeles, Los Angeles, CA, USA.

BACKGROUND: Current autologous tissues used for urethral reconstruction, such as prepuce or buccal mucosa, lack the mechanical and structural properties of the multilayered anterior urethra. In our previous work, we demonstrated the feasibility of creating a nanofibrous synthetic scaffold with target tensile properties derived from the native urethra (HIS 2023). This study aim was to evaluate these candidate materials in vitro and in vivo to assess cytocompatibility and biocompatibility, respectively.

Methods: Nanofibrous scaffold sheets were synthesized by electrospinning two naturally derived biomaterials, 5% ELP (elastin like peptide)/5% GelMA (gelatin methacryloyl) weight per volume (w/v, 5E5G) or 10% w/v GelMA alone (0E10G), selected for tensile matched properties with urethra based on our prior characterization work (HIS, 2023). Human bladder- and foreskin-derived cell lines (ATCC) were seeded onto scaffolds in monoculture conditions (1x10⁶ cells/mL). Cell viability, adhesion/spreading, cytotoxicity and proliferation were evaluated at day 1 to 7 endpoints. Biocompatibility testing subsequently evaluated tissue regeneration, degradation, and immune response in Sprague-Dawley rats for 4 subcutaneous cohorts: 1) sham, 2) 5E5G, 3)0E10G and 4) one-ply small intestinal submucosa (SIS, Cook), assessed at day 1 to 56 endpoints.

Results: 5E5G and 0E10G nanofibrous scaffolds demonstrated excellent cell viability from day 1 to 7 across urothelial (UC), smooth muscle (SMC) and fibroblast (Fib) cell lines. Proliferation and spreading of SMCs and Fib was robust, while UCs demonstrated less scaffold adherence and proliferation than the other two cell lines. Furthermore, addition of ELP ensured enhanced extensibility, degradation, tissue ingrowth and minimized acute or chronic inflammation in biocompatibility testing.

Conclusion:

Highly elastic engineered GelMA/ELP hybrid scaffolds are non-cytotoxic and biocompatible. Future scaffold modifications are ongoing to optimize parameters for UC proliferation. Additionally, our team is utilizing these materials in bioinks and creation of multi-layered 3-dimensional structures with optimized microenvironments by cell line to enhance location-specific regeneration.



Evaluating Ultrasonographic Measurements of Fetal External Genital Morphology: A Systematic Review of Techniques and Normative Values

Authors: Nikit Venishetty BA, Ishant Goel, Akash Chauhaun, Maral Demirjian MPH, Lorna Kwan MPH, Renea Sturm MD FAAP

Affiliation: David Geffen School of Medicine, University of California Los Angeles, Department of Urology

Introduction/Aim: Fetal ultrasound measurements of external genitalia lack standardizations and specificity for diagnosing hypospadias and other postnatal genitourinary pathology. The aim of this systematic review was to investigate variability in sonographic measurement methods in current literature and to establish normative values and growth curves for fetal genitalia measurements.

Methods: A literature review was completed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Three database sources were included: Pubmed, Google Scholar, and Web of Science. Studies were included that reported sonographic fetal penile length, width, and/or anogenital distance measurements by gestational age. Exclusion criteria included studies evaluating other imaging modalities (e.g., MRI), or only fetuses with systemic anomalies (e.g., intrauterine growth restriction). Case reports, reviews, commentaries, and non-English studies were also excluded. All measurements were grouped by measurement location (e.g., cavernosal vs penoscrotal length). Data was standardized to the mean (mm) and 95% confidence interval, then plotted over gestational age.

Results: Our search criteria yielded 173 articles, of which 17 were included in the analysis. Eight evaluated fetal penile length from the scrotum to the tip of the penis, 6 penile width at the widest point of the shaft, and 3 anogenital distance. The aggregate data was applied to establish a reference equation for the overall mean (+ 95% CI) penile length and width in normal fetal development (**Figure 1 and 2**). Adequate data was not available for evaluation of predictive anatomic parameters associated with anomalous postnatal penile development.

Discussion and Conclusion: The growth curves from this review will provide a valuable reference for clinicians assessing penile length, width, and anogenital distance in fetal ultrasounds, facilitating comparison to normative measurements by gestational age. Future collaborative efforts are needed to establish standardized measurement approaches and to evaluate the predictive value of sonographic findings for hypospadias or other genital developmental differences.