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Editorial for the Proceedings of the 5th Pediatric Andrology Meeting on Cryptorchidism

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Editorial for the Proceedings of the 5th Pediatric Andrology Meeting on Cryptorchidism

Valletta, Malta — 26–27 September 2025

We are pleased to introduce the proceedings of the **5th Pediatric Andrology Meeting on Cryptorchidism**, held from **Friday, September 26 to Saturday, September 27, 2025**, at the International Meeting Center in Valletta and conducted simultaneously via a hybrid Zoom platform. Over its two days, the meeting once again fulfilled its central mission: **to provide a forum for the exchange of ideas, for the presentation of new data, and for strengthening interactions among clinicians, surgeons, endocrinologists, and molecular scientists** working to improve care for boys with cryptorchidism.

Bringing together experts from **molecular biology, pediatric endocrinology, pediatric surgery, and andrology**, the 2025 symposium offered a comprehensive view of current advances in diagnosis, mechanisms, and treatment. Discussions repeatedly emphasized that cryptorchidism must be understood as **more than a simple failure of testicular descent**. Instead, it represents a condition shaped by endocrine programming, germ-cell biology, molecular architecture, and increasingly recognized, **neurocognitive factors**.

One particularly striking theme emerging from both the in-person and virtual sessions was the **importance of hormonal treatment not only for testicular maturation but also for potential improvements in memory and cognition in cryptorchid boys**. These observations broaden the conceptual framework of cryptorchidism beyond an anatomic disorder and reinforce the view that early disturbances of the hypothalamic–pituitary–gonadal axis carry systemic developmental implications.

The central focus of the symposium was the **integration of molecular biology with pediatric endocrine practice**. Several talks delineated how **PIWIL and TUDOR gene families**, essential for germline genome protection and piRNA biology, may play decisive roles in shaping fertility potential in boys with undescended testes. These discussions highlighted how translational genetics is reshaping our understanding of the germ-cell consequences of cryptorchidism.

Equally compelling were the contributions examining **epigenetic mechanisms and the role of estrogenic signaling** in the origin of cryptorchidism. Presentations highlighted how endocrine and epigenetic perturbations during fetal life and mini-puberty can disrupt the transformation of gonocytes and interfere with Sertoli- and Leydig-cell maturation. These findings underscore a broader principle that **hormonal and epigenetic disruptions underlie the etiology of many apparently mechanical cases of undescended testes**.

Another notable topic was the emerging evidence regarding the **cystic fibrosis factor and its involvement in fertility development**. The symposium examined how this factor responds

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negatively to a hypogonadotropic state in cryptorchid boys at high risk for infertility, adding a new dimension to the classification of biological vulnerability and demonstrating the expanding reach of molecular diagnostics.

These molecular, endocrine, and epigenetic insights converged on a significant clinical conclusion: **the aberrations identified cannot be corrected by early surgery alone**. While timely orchiopexy remains essential for anatomic repositioning and long-term surveillance, it does not address upstream defects arising from mini-puberty disturbances, hormonal insufficiency, or molecular instability. Consequently, the meeting's interdisciplinary consensus was that **hormonal therapy—particularly GnRH agonists or hCG—should be strongly considered as part of treatment guidance**, especially for boys exhibiting biological markers of high infertility risk. Incorporating endocrine induction into management pathways was viewed not as experimental, but as a logical evolution of mechanism-based care.

The preparation of these proceedings benefited from **OpenAI's Deep Research tool**, used exclusively for **copy-editing, proofreading, and improving linguistic clarity and organization**. Its application did **not** generate new scientific or conceptual content; every scientific idea herein remains the original work of the contributing authors. Each manuscript included in this volume has been **carefully reviewed for factual accuracy and approved by its author(s) and by the editorial team**.

We hope that this collection reflects the depth, rigor, and collaborative spirit of the 2025 meeting. As cryptorchidism continues to be redefined through advances in molecular biology, endocrinology, and developmental science, it is our conviction that meetings such as this—and the shared knowledge they generate—will play a critical role in shaping the next generation of evidence-based, mechanistically informed care for affected boys worldwide.

Editors

Proceedings of the 5th Pediatric Andrology Meeting on Cryptorchidism
Valletta, Malta — 2025em

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The 5th International Andrology Symposium Cryptorchidism

Faruk Hadziselimovic(Liestal):**Welcome address**

Molecular biology 1.
Chair: **Mizuno Kentaro** (Nagoya) **Antoine Peters** (Basel)
Vincent Prevot (Lille): Molecular biology of the mini-puberty, LH-RH and cognition
Rodolfo Ray (Buenos Aires)**:**The physiology of the transient postnatal activation of the hypothalamic-pituitary-testicular axis
Antoine Peters (Basel)**:** Epigenetic gene regulation in male germ cell development.
Birgit Stallmeyer (Münster): Impact of disturbed piRNA biogenesis on transposon de-repression and male infertility

Molecular biology 2.
Chair: **Gilvydas Verkauskas** (Vilnius) **Jorma Toppari** (Turku)
Kentaro Mizuno (Nagoya)**:** Biological effects on early-stage spermatogenesis in cryptorchidism
Jorma Toppari (Turku): Puberty in boys with a history of cryptorchidism

Shosei Yoshida (Okazaki): Roles of temperature and retinoic acid in the spermatogenesis defect associated with cryptorchidism.
Christian De Geyter (Basel): Altered DNA methylation in estrogen-responsive repetitive sequences of spermatozoa of infertile men with shortened anogenital distance

Pathophysiology
Chair: **Rodolfo Ray** (Buenos Aires) **Faruk Hadziselimovic** (Liestal)
Gilvydas Verkauskas (Vilnius)**:**Hypothalamus-pituitary-gonadal axis in cryptorchid boys
Darius Dasevicius (Vilnius)**:**Histopathology of cryptorchid tests.
Dina Cortes (Copenhagen): Negative effect of hormonal treatment on prepubertal testis
Jörgen Thorup (Copenhagen): Testis tissue cryopreservation may be considered for boys with cryptorchidism.

Treatment
Chair: **Guy Bogaert** (Leuven) **Dina Cortes** (Copenhagen)
Bica Domingos (Rio de Janeiro): Buserelin-treatment; three-arm placebo-controlled study.

Dimitrios Papadimitriou (Athens): Replacement of male mini-puberty"

Faruk Hadziselimovic(Liestal): Molecular evidence supports hormonal treatment

Luciano Favorito (Rio de Janeiro): The role of gubernaculum in testicular migration - translational aspects applied to undescended tests

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Round Table Discussion & Videoconferencing to address specific topics and recent advancements and questions by the audience.

Moderators: **Guy Bogaert**(Leuven, **Gianantonio Manzoni**(Milano)

- Pitfalls in cryptorchidism diagnosis**. Dragana Zivkovic**(Novi Sad)
- Epidydimo-testicular descent. **Faruk Hadziselimovic**(Liestal)
- Advantages and disadvantages of early orchidopexy. **Zacharias Zachariou**(Nicosia)
- Benefits and drawbacks of hormonal treatment/orchidopexy). **Guy Bogaert**(Leuven)
- Is the current guidance for the clinical management of cryptorchidism up-to-date and based on the latest research findings? **Beata Vincel** (Vilnius)

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Molecular Biology of Mini-Puberty, LH-RH and Cognition

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Abstract

Mini-puberty—the brief, physiological re-activation of the hypothalamic–pituitary–gonadal (HPG) axis in early infancy—is a process critical for adult male and female reproduction. Work led by Prévot and colleagues defines mini-puberty as a neurodevelopmental program that determines both reproductive and cognitive functions. At its core is a molecular switch in infant GnRH (LH-RH) neurons in which microRNAs (notably the miR-200 family and miR-155) repress transcriptional repressors (e.g., ZEB1 and C/EBPβ), enabling the postnatal upregulation of *GNRH1* transcription. Nitric-oxide (NO) signaling *via* NOS1 modulates this switch and therefore influences GnRH signaling. Perturbations—including DICER loss in GnRH neurons, trisomy-driven microRNA disequilibria in Down syndrome models, or NOS1 deficiency—distort pulsatility, a critical feature of the GnRH signal, with downstream reproductive and cognitive consequences. Strikingly, pulsatile (but not continuous) GnRH delivery improves cognition and strengthens basal network connectivity in adults with Down syndrome, extending GnRH's role from a purely reproductive hormone to a systems-level neuromodulator of cortical networks. These mechanistic insights intersect directly with andrology: mini-puberty determines androgen/INSL3 function during normal testicular descent, while absent or distorted infantile HPG dynamics are linked to abnormal genital development (micropenis) and cryptorchidism in congenital GnRH deficiency. Here, I describe the development of GnRH neurons, molecular biological insights into the infantile *GNRH1* transcriptional switch, evidence for extra-hypothalamic GnRH actions on cognition, and clinical implications for cryptorchidism and pediatric endocrinology.

Key words Mini-puberty, GnRH neuron, Down syndrome, memory

Résumé

La mini-puberté—réactivation physiologique transitoire de l'axe hypothalamo–hypophyso–gonadique (HPG) en début de vie—est essentielle à la reproduction ultérieure chez l'homme et la femme. Les travaux de Prévot et collaborateurs en font un programme neurodéveloppemental déterminant les fonctions reproductives et cognitives. Au cœur de ce processus se trouve un commutateur moléculaire dans les neurones GnRH du nourrisson : des microARN (notamment la famille miR-200 et miR-155) répriment des répresseurs

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transcriptionnels (ZEB1, C/EBPβ), permettant l'activation postnatale de la transcription de **GNRH1**. La signalisation du monoxyde d'azote (NO), via **NOS1**, module ce commutateur et donc l'activité GnRH.

Toute perturbation—perte de **DICER** dans les neurones GnRH, déséquilibres microARN induits par la trisomie dans les modèles de syndrome de Down, ou déficit en **NOS1**—altère la pulsatilité du signal GnRH, caractéristique indispensable à ses effets reproductifs et cognitifs. De manière remarquable, une administration **pulsatile**, mais non continue, de GnRH améliore la cognition et la connectivité corticale de base chez l'adulte trisomique, étendant le rôle de GnRH d'une hormone strictement reproductive à celui de neuromodulateur des réseaux corticaux.

Ces avancées éclairent directement l'andrologie : la mini-puberté régule la fonction androgène/INSL3 dans la descente testiculaire, tandis que l'absence ou la distorsion de l'activation HPG infantile est liée au micropénis et à la cryptorchidie dans les déficits congénitaux en GnRH. Cette revue décrit le développement des neurones GnRH, les mécanismes moléculaires du commutateur transcriptionnel infantile de **GNRH1**, les actions extra-hypothalamiques de GnRH sur la cognition et leurs implications cliniques pour la cryptorchidie et l'endocrinologie pédiatrique.

Mots-clés: Mini-puberté, neurone GnRH, syndrome de Down, mémoire

Developmental background

GnRH neuron differentiation. A few thousand GnRH (LH-RH) neurons originate in the olfactory placode, migrate into the forebrain, then project to the median eminence where pulsatile GnRH release drives pituitary LH/FSH secretion in a process conserved across mammals [1,2,3,4]. Beyond the canonical hypothalamic population, an extra-hypothalamic type of *GNRH1*-expressing neurons has been identified in adult human basal ganglia and the basal forebrain, many with a cholinergic phenotype, expanding plausible brain targets of GnRH signaling [4, 5]**.** The GnRH signal profile is comprised of fetal activation, mini-puberty (occurring several weeks after birth in humans), juvenile quiescence, pubertal re-activation, and senescent decline. Importantly, the biological effect depends on the frequency and amplitude of signal pulses to pituitary and brain targets rather than transmitter levels [6].

Mini-puberty: timing, sex differences, and significance

In term human infants, LH/FSH rise in the first weeks, peak between 1–3 months, and decline from around six months onward in boys; FSH can remain comparatively elevated for longer periods of time in girls [2]. This period promotes Sertoli cell proliferation, Leydig cell steroidogenesis, ovarian signaling and determines penile growth and anogenital distance, thereby likely establishing adult reproductive capacity [2]. In rodents, these processes occur during the second–third postnatal weeks, scaling with lifespan.

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Mini-puberty represents with the last developmental step where spontaneous testicular descent occurs; endocrinological data obtained during this period can inform prognosis in cryptorchid boys[3].

The infant *GNRH1* transcriptional switch: microRNAs and NO

microRNA control of *GNRH1* expression. In infant GnRH neurons, microRNAs contribute to the regulation of *GNRH1* expression after birth. Genetic disruption of microRNA biogenesis (e.g., conditional *DICER* mutation in GnRH neurons) leads to progressive loss of GnRH peptide despite neuronal survival and culminates in infertility—evidence that postnatal gene-regulatory control is essential for maintaining the GnRH phenotype (summarized from the provided transcript and supported by microRNA-puberty reviews) [7].

NO/NOS1 as a permissive modulator. NO signaling contributes to this switch's kinetics. Rare *NOS1* variants in humans cause congenital hypogonadotropic hypogonadism (CHH) with sensory and cognitive features. In mice, loss of NOS1 exaggerates mini-puberty hormone profiles and impairs sensory–cognitive behaviors in adults, which can be rescued in mice by transient NO-pathway stimulation (inhaled NO or sildenafil) during the infantile period [8]. These data reveal NO as a modulator of infant GnRH activation.

Trisomy-21-dependent disequilibria (Down syndrome). In the Ts65Dn mouse model of Down syndrome, GnRH transcripts fall at the peak of rodent mini-puberty, with up-regulation of ZEB1 (consistent with reduced miR-200 levels); in adulthood, GnRH peptide diminishes and LH pulsatility shifts to higher baseline with blunted peaks **without** change in testosterone—an endocrine signature also observed in adult men with Down syndrome [1]. Together with human imaging and cognitive data (Section 5), these findings support a link between early molecular tuning of GnRH neurons during the infantile period and altered brain and endocrine network function in adulthood.

Pulsatility is the message— and it reaches the cortex

Anatomy to mechanism. Tissue-clearing and tract-tracing work (as presented in the transcript) and human histological mapping indicate GnRH-responsive elements outside the hypothalamus, including cortex, hippocampus, and basal ganglia [1,4,8]. This provides a substrate for GnRH as a neuromodulator of attention, memory, and network synchrony.

Continuous vs pulsatile delivery. In adult wild-type mice, continuous GnRH delivery suppresses the HPG axis by desensitizing the GnRH receptor (GnRH-R) on pituitary gonadotrophs, but also impairs cognition by producing the same receptor desensitization in GnRH-R-expressing neocortical neurons in the brain [1]. However, pulsatile GnRH delivery restores olfaction and cognition in trisomic mice [1]. Clinically, neurocognitive complaints during long-term GnRH-agonist therapy for sex-steroid-dependent conditions are consistent with the hazards of desensitizing, non-physiologic stimulation. However, this remains hypothesis-generating rather than proof of causality and would require further clinical studies in humans to be confirmed. Conversely, pulsatile GnRH has decades of safety/efficacy in

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CHH/functional hypothalamic amenorrhea and now shows cognitive benefits in Down syndrome (next section).

Human translation: pulsatile GnRH enhances cognition in Down syndrome

In a pilot open-label study, seven adults with Down syndrome received pulsatile GnRH (1 pulse/2 h) for six months via a portable pump. All improved on composite cognitive measures (~20% mean), and resting-state functional magnetic resonance imaging (fMRI) showed stronger default-mode network connectivity—an objective network shift in a system typically hypoactive in Down syndrome [1]. Commentaries highlight the novelty and call for controlled trials [9,10]. While preliminary, these results suggest GnRH to be not only critical for reproduction but also systems-level neuromodulation with therapeutic implications.

Cryptorchidism and the critical role of mini-puberty

Two-phase model and molecular drivers. The transabdominal phase of descent is driven largely by INSL3 acting via RXFP2, whereas the inguinoscrotal phase requires androgens (testosterone/DHT) and neural inputs [11-13]. Human genetics work identified biallelic loss-of-function variants in INSL3 or RXFP2 that cause bilateral cryptorchidism and infertility, with heterozygous carriers often unaffected—underscoring the pathway's central role in the process [11].

Essential functions of mini-puberty. Mini-puberty supplies the androgen/INSL3 environment in the first six months to enable spontaneous descent. Prospective observations suggest some undescended testes descend during this period; persistence beyond approximately six months indicates the need for surgery [3]. Hormone ratios (e.g., LH/testosterone or LH/INSL3) during mini-puberty may be more informative for Leydig cell efficiency than absolute values and the prognosis of ultimate testicular descent [3,1,13].

CHH and absent mini-puberty. In congenital GnRH deficiency, the infant LH/FSH surge is attenuated or absent, predisposing patients to micropenis**,** cryptorchidism, and impaired testicular maturation—mirrored by mouse models where selective impairment of GnRH release (with preserved migration) yields cryptorchidism, micropenis, reduced anogenital distance, and absent LH pulsatility (from the transcript; clinical analogs summarized in [2,3].

Prematurity, exaggerated mini-puberty, and NO-pathway therapeutics

Preterm infants often display abnormally high gonadotropin profiles in mini-puberty, and they carry higher risks for later neurocognitive and metabolic issues. Experimental **NOS1** loss exaggerates mini-puberty in mice and impairs cognition, but short-term inhaled NO or sildenafil treatment of infantile mice normalizes endocrine signatures and rescues adult

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behaviors [8]. Since these agents are already used in neonatal pulmonary care and show no adverse effects on later cognitive outcomes [14]. pursuing a translational program with time-specific NO supplementation is a plausible path (with careful ethics and endpoints).

Therapeutic implications

Respect pulsatility. Where GnRH therapy is indicated, pulsatile delivery preserves physiological signaling. In CHH/functional hypothalamic amenorrhea, pulsatile GnRH remains a gold-standard option; in Down syndrome and possibly other neurodevelopmental contexts, pulsatile GnRH emerges as a candidate neurotherapeutic under clinical trials [1,2].

Mini-puberty replacement in CHH infants. Early gonadotropin therapy to mimic mini-puberty (rhLH/FSH via pump or injections) promotes penile growth, Sertoli cell proliferation, and testicular descent in CHH boys, strengthening the case for time-sensitive endocrine replacement alongside surgical intervention [2,15-17].

Pathway-specific adjuncts. In the case of cryptorchidism caused by INSL3/RXFP2 defects, endocrine therapies will not restore a dysfunctional receptor–ligand axis, underscoring the priority of timely orchiopexy; however, understanding whether an individual's clinical state is androgenic vs INSL3-centric may influence prognosis of fertility and follow-up care [11,13]. In prematurity-associated dysregulation, NO-pathway modulation in infants is an attractive research avenue [8].

Practical checklist for clinicians (0–6 months)

1. **Profile dynamics, not just levels.** Consider LH, testosterone, INSL3**,** and ratios (LH/testosterone, LH/INSL3) when evaluating cryptorchid infants; integrate with gestational age and clinical exam [3,12,13].
2. **Consider CHH when mini-puberty is absent.** Micropenis ± cryptorchidism with low/flat LH/FSH/testosterone suggests CHH and justifies early endocrine and surgical coordination [2,3].
3. **Use pulsatile patterns when treating with GnRH.** Avoid flattening the axis unless clinically necessary; anticipate potential neurocognitive consequences of long-term continuous GnRH-agonist therapy (hypothesis based on animal and clinical observation) [1,6].
4. **Discuss trials.** For selected contexts (e.g., Down syndrome cognition, prematurity-related dysregulation), consider referral to research studies exploring pulsatile GnRH **or** NO-pathway interventions [1,8].

Conclusions

Mini-puberty is a developmental process that affects both reproductive and cognitive systems. A microRNA–transcription factor program, responding to NO signaling, operates a

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transcriptional switch in *GNRH1* expression in infants and sets pulsatile dynamics of GnRH signaling. Disruptions (microRNA imbalance, NOS1 variants, trisomy-driven disequilibria, or non-physiologic therapies) can simultaneously skew reproductive outcomes (micropenis, cryptorchidism, later infertility risks) and degrade cognition. The demonstration that pulsatile GnRH improves cognition and network connectivity in adults with Down syndrome reveals GnRH as a brain-wide neuromodulator and opens a translational path for developmental stage-specific interventions. Combining endocrinological exams and timely surgery during mini-puberty is a promising approach to treating cryptorchidism and adult infertility it entails.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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The physiology of the transient postnatal activation of the hypothalamic–pituitary–testicular axis

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Abstract

The male hypothalamic–pituitary–testicular (HPT) axis is activated three times across the life course: during fetal life, transiently in early infancy (mini-puberty), and again with the onset of true puberty. Although both mini-puberty and puberty feature hypothalamic–pituitary activation, mini-puberty is a **developmentally distinct** program: Leydig cells secrete testosterone and INSL3, Sertoli cells proliferate and secrete AMH and inhibin B, but spermatogenesis does not start because Sertoli cells are physiologically androgen-insensitive in this window. The first trimester of fetal life is unique in that masculinization is **pituitary-independent** and driven by placental hCG acting on LHCGR; by contrast, from mid-gestation onward and postnatally, pituitary control predominates. In this proceedings contribution, I synthesize cellular ontogeny (Leydig, Sertoli and germ cells), endocrine dynamics (LH/FSH, testosterone, INSL3, AMH, inhibin B), and clinical correlates (cryptorchidism, micropenis, congenital hypogonadotropic hypogonadism), integrating new molecular and single-cell insights. Mini-puberty is clinically invaluable: it offers a diagnostic window for central hypogonadism, explains phenotypic differences according to timing of androgen insufficiency, and informs management of undescended testes. Physiologic replacement strategies that **mimic mini-puberty** in boys with congenital hypogonadotropic hypogonadism (CHH) are emerging and appear to improve penile growth, Sertoli-cell output, and sometimes testicular descent. Finally, I frame practical implications for testing and timing of orchiopexy and articulate research priorities linking early endocrine programming to later fertility.

Key words Mini-puberty, fetal life ,gonadotropins

Résumé

L'axe hypothalamo–hypophyso–testiculaire (HPT) masculin s'active à trois périodes de la vie : pendant la vie fœtale, transitoirement au début de la vie (« mini-puberté ») et à l'adolescence lors de la puberté. Bien que mini-puberté et puberté impliquent une activation hypothalamo–

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hypophysaire, la mini-puberté constitue un programme développemental distinct : les cellules de Leydig sécrètent testostérone et INSL3, les cellules de Sertoli prolifèrent et sécrètent AMH et inhibine B, mais la spermatogenèse ne débute pas en raison de l'insensibilité physiologique des cellules de Sertoli aux androgènes dans cette fenêtre. Au premier trimestre fœtal, la masculinisation est indépendante de l'hypophyse et dépend de l'hCG placentaire agissant sur LHCGR ; à partir du milieu de la gestation et en période postnatale, le contrôle hypophysaire devient prépondérant. Cette contribution synthétise l'ontogénie cellulaire (Leydig, Sertoli, cellules germinales), les dynamiques endocriniennes (LH/FSH, testostérone, INSL3, AMH, inhibine B) et leurs corrélats cliniques (cryptorchidie, micropénis, hypogonadisme hypogonadotrope congénital), en intégrant de nouveaux éclairages moléculaires et unicellulaires. La mini-puberté offre une fenêtre diagnostique cruciale pour l'hypogonadisme central, explique les phénotypes selon le moment de l'insuffisance androgénique et guide la prise en charge des testicules non descendus. Des stratégies de remplacement physiologique mimant la mini-puberté émergent chez les garçons atteints de CHH et améliorent la croissance pénienne, la fonction sertolienne et parfois la descente testiculaire. Enfin, sont proposées des implications pratiques pour les tests hormonaux, le moment optimal de l'orchidopexie et les priorités de recherche reliant la programmation endocrine précoce à la fertilité future.

Mots-clés: Mini-puberté, vie fœtale, gonadotrophines

Introduction

The HPT axis follows a multi-phase developmental choreography. The **first trimester** is dominated by placental **hCG** acting on the shared **LH/CG receptor (LHCGR)** to drive fetal Leydig-cell steroidogenesis underlying male genital differentiation—**independently** of fetal pituitary function. In the **second and third trimesters**, fetal pituitary LH/FSH increasingly contribute to testicular function. Shortly after birth, and following a brief suppression during the first days of life, the axis is **reactivated** for several months: LH and FSH rise, Leydig products (testosterone and **INSL3**) peak, and Sertoli-cell markers (**AMH**, **inhibin B**) are conspicuously elevated. Then the axis becomes quiescent until **true puberty**, when GnRH pulsatility resumes and spermatogenesis begins [1]. The contribution to the meeting emphasized these contrasts and their clinical consequences: **ambiguous genitalia** if androgen insufficiency occurs in the **first trimester**, versus **micropenis and cryptorchidism** (without ambiguity) if hypogonadism arises later; Leydig cells in childhood are hard to stimulate with a **single** LH/hCG dose; and Sertoli cells, although active, are **immature** and lack androgen receptor (AR) function in infancy, explaining high AMH and absent spermatogenesis.

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Fetal life, placental hCG, and the timing principle

First trimester: masculinization under placental control

In the first trimester, **placental hCG** engages **LHCGR** on fetal Leydig cells to stimulate testosterone (and downstream DHT) needed for **male external genitalia** and urogenital tract masculinization. Because this step is **pituitary-independent**, defects in fetal pituitary GnRH/LH will **not** cause undervirilization; instead, defects in **LHCGR** function or androgen synthesis/action will [2]. Clinically, therefore, in 46,XY fetuses, **ambiguous genitalia/DSD** point upstream to placental/Leydig/androgen pathways rather than central hypogonadism when the insult occurs in the fi trimester.

Second–third trimesters: increasing pituitary contribution

From mid-gestation onward, the fetal pituitary becomes an active partner. Hypogonadism arising in this window or **postnatally** will usually **spare virilization** (already completed under hCG drive) yet predispose to **micropenis** and **cryptorchidism** because penile growth, testicular descent (and Leydig trophism) depend on later **androgen** and **INSL3** output. The distinction by **timing** explains divergent phenotypes seen in clinic and underpins rational work-ups [3-5]

Cellular ontogeny across epochs

Leydig cells: fetal/infantile vs adult populations

Fetal (and early infantile) Leydig cells are hCG/LH-responsive, produce **testosterone** and **INSL3**, and regress during later infancy/childhood; **adult** Leydig cells emerge at puberty. INSL3, an RLF/insulin superfamily peptide, reflects Leydig-cell **functional capacity** and displays a characteristic **postnatal peak** (~2–3 months), falls to near-undetectable levels through childhood, and rises again across puberty. Longitudinal and cross-sectional studies position INSL3 as a robust **biomarker** complementing testosterone, with distinct kinetics [6-8].

Functional stimulation. In the quiescent years, a **single** hCG/LH injection yields a **blunted** testosterone response; repeated stimuli or physiologic **pulsatility** better reveal Leydig capacity—consistent with clinical observations and with the idea that central hypogonadism (never exposed to LH in utero/mini-puberty) leaves Leydig cells even less responsive [9].

Sertoli cells: proliferation and endocrine signatures

Sertoli cells proliferate during fetal life, **mini-puberty**, and childhood under **FSH** influence; they secrete **AMH** and **inhibin B** and remain **immature** until puberty [10]. A cardinal mechanism explains why spermatogenesis does not start in infancy despite high testosterone: in early postnatal life, **Sertoli cells lack androgen receptor (AR)**, so AMH remains **high** (androgen-repressible only after AR expression at later ages), seminiferous tubules remain small, and meiosis/spermiogenesis do not occur [11].

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AMH and inhibin B thus serve as **Sertoli biomarkers**: AMH is high from fetal life through childhood, falls with pubertal AR activation; inhibin B is measurable and tracks Sertoli number/function. These insights power pediatric diagnosis where testosterone may be uninformative, and they are central to distinguishing selective Leydig defects from **global** testicular failure [1].

Germ cells: from PGCs to spermatogonia—what single-cell maps add

Primordial germ cells (PGCs) migrate to the genital ridges, differentiate into **gonocytes/prospermatogonia**, then **spermatogonia**. Single-cell RNA-seq atlases across fetal, neonatal and adult testes now chart molecular states of **Sertoli**, **Leydig**, **peritubular**, and **germ-cell** populations, clarifying that neonatal testes host undifferentiated SSC-like pools and prospermatogonia that do **not** enter meiosis until puberty. These datasets provide the cellular scaffold for interpreting endocrine signals across mini-puberty and puberty [7].

The transient postnatal activation (mini-puberty)

Temporal profile in term boys

In human males, the HPT axis is transiently **suppressed at birth** (first 1–2 days), then **activates**: **LH/FSH** rise within the **first week [12].** peaking around **1–2 months [13].Testosterone** and **INSL3** follow the LH peak by several weeks. **Sertoli** outputs are also robust: **AMH** stays high; **inhibin B** is elevated in early infancy and declines partially thereafter but remains measurable—unlike testosterone, which falls to very low/undetectable levels through mid-childhood. (Oxford Academic)

During mini-puberty, **testicular volume** increases about **2–3-fold**, predominantly from **Sertoli proliferation** and **seminiferous-tubule lengthening** (not diameter) [14]. This has methodological implications: cross-sectional histologic counts may paradoxically show fewer Sertoli cells per cross-section even when **total** Sertoli number is rising, simply because cells are distributed along longer tubules. Ultrasound is superior to orchidometry for precise testicular volume estimates in this window [15].

Preterm vs term infants: corrected age matters

Preterm boys demonstrate **higher and more prolonged** LH/FSH and testosterone profiles and a later decline than term boys; however, when indexed to **corrected gestational age**, levels converge. The axis thus appears to follow an **intrinsic developmental clock** rather than chronological time—a crucial nuance for interpreting laboratory results and deciding when to investigate [16,17].

Predictive value

Observational cohorts suggest that **testosterone** levels during mini-puberty correlate with later **sperm counts** in adulthood—consistent with the concept that the infant surge tunes later testicular capacity, though effect sizes and confounding require more study [18]. **INSL3**

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may emerge as a complementary predictor, given its reflection of Leydig cell **functional mass**, but longitudinal validation is ongoing [19].

Why mini-puberty is not puberty

Despite shared features (GnRH/LH/FSH activation), mini-puberty **lacks** the Sertoli-cell androgen responsiveness required for spermatogenic **maturation** [20]. AR is **not** expressed in Sertoli cells during early human testis development, explaining persistent **high AMH** and absence of meiosis/spermiogenesis [21]; with puberty, intratesticular testosterone rises, AR is expressed in Sertoli (and peritubular) cells, **AMH** falls, inhibin B rises further, and full spermatogenesis begins. This developmental staging clarifies why robust infant testosterone does **not** trigger puberty and why testicular volume growth at this age reflects **Sertoli proliferation** rather than germ-cell mass [22].

Clinical mapping by timing of hypogonadism

First trimester androgen pathway defects (DSD)

Defects in androgen **synthesis/action** or **LHCGR** during the **first trimester** cause **ambiguous genitalia/DSD** because masculinization is hCG/LHCGR-dependent in that window and **pituitary-independent**. AMH is **present** (Sertoli-derived) and useful to assess Sertoli activity and Müllerian regression, distinguishing primary gonadal failure from androgen-pathway defects in the DSD work-up [2].

Mid-gestation/postnatal central hypogonadism (CHH)

Central defects (GnRH/LH/FSH deficiency) arising **after** first-trimester virilization typically present **without** genital ambiguity but with **micropenis** and **cryptorchidism** due to insufficient later testosterone/INSL3. In **CHH**, mini-puberty is **absent or blunted**: LH/FSH/testosterone are low, AMH/inhibin B lower than age norms, and testicular volume small. Recognizing this pattern in the **1–6-month** window enables **early diagnosis** and appropriately timed **physiologic replacement [23-26].**

Cryptorchidism: endocrine window, biomarkers, and surgery

Testicular descent comprises a **transabdominal** phase (largely **INSL3–RXFP2**-driven) and an **inguinoscrotal** phase (androgen-dependent). Mini-puberty supplies critical **androgen/INSL3** trophism in the final window for **spontaneous** descent (3). If the testis has **not** descended by approximately **6 months** (corrected for gestational age), spontaneous descent thereafter is **unlikely [27].**

Biochemical evaluation may include **LH, FSH, testosterone, INSL3, AMH, and inhibin B**. Patterns and **ratios** (e.g., LH/testosterone, LH/INSL3) can reveal Leydig inefficiency even

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when single analytes overlap reference intervals. **AMH/inhibin B** help differentiate global testicular dysfunction (low) from selective Leydig failure (AMH/inhibin B preserved) [28,29].

Inducing mini-puberty in CHH: proof-of-concept and practice

Given the developmental rationale, **physiologic replacement** to **mimic mini-puberty** in boys with **CHH** has been explored using **recombinant LH/FSH** (via **pumps** or **injections**) or **hCG/FSH** combinations [23,25,30]. Across case series and small cohorts, therapy during the first months of life increases **penile length**, **testicular volume**, and **Sertoli outputs** (AMH, inhibin B) and can assist **testicular descent** in some cases. Protocols vary in dosing and duration, and long-term fertility outcomes require careful follow-up, but accumulating evidence supports the **safety** and **physiologic logic** of time-locked gonadotropin replacement when mini-puberty is missing.

Practical points. Replacement is ideally delivered in specialized centers; **monitoring** includes LH/FSH (if endogenous), testosterone, **INSL3**, **AMH**, **inhibin B**, penile length, and testicular volume by ultrasound. Because Leydig responsiveness in childhood requires **repeated** stimulation, **pulsed** or **recurrent** dosing regimens are physiologically sound.

Quantitative features of mini-puberty hormones

LH/FSH

LH and FSH rise within the **first week**, peak around **1–2 months**, and decline to low levels by **4–6 months**. **FSH** tends to remain relatively higher than LH across childhood, reflecting persistent Sertoli activity [31]. These patterns must be interpreted with **age-appropriate** (and gestation-corrected) reference intervals.

Testosterone

Serum testosterone (T) peaks **after** the LH surge and then **falls** to very low levels through childhood [31]. Importantly, **intratesticular** testosterone at early puberty may be high even when serum levels still appear **prepubertal**—a key reason why spermatogenesis can initiate **before** marked serum T elevation in adolescence [32].

INSL3

INSL3 peaks around **3 months**, then becomes **low/undetectable** in mid-childhood, rising again with **pubertal** Leydig differentiation [33,34]. As a relatively **stable** secretion reflecting Leydig mass, INSL3 may be less acutely variable than testosterone and thus useful when assessing Leydig capacity longitudinally.

AMH and inhibin B

AMH is **high** from fetal life through childhood, declining at puberty when androgens repress Sertoli transcription via AR [29]; **inhibin B** is measurable throughout infancy and childhood

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and rises further at puberty with germ-cell expansion [35]. Together, they provide a **Sertoli-centric** window into the testis when LH/testosterone are uninformative.

Methodological considerations

Assay and sampling caveats

Neonatal and infant assays require **sensitive** platforms and strict pre-analytical control (timing, posture, illness). For **INSL3**, LC-MS/MS and validated immunoassays with pediatric reference data are preferred; for **AMH/inhibin B**, harmonization across platforms is improving but inter-assay variation persists. Clinicians should rely on **laboratory-specific** pediatric reference intervals and consider **corrected gestational age** in preterm infants.

Imaging and volumetry

Ultrasound-based ellipsoid models outperform orchidometry in small testes and are sensitive to the **2–3-fold** volume increase observed during mini-puberty [15]. Given seminiferous tubule **lengthening** rather than diameter expansion, histologic quantification should avoid naïve cross-sectional counts as proxies for Sertoli number [14,22].

Integrating single-cell genome biology with endocrine dynamics

Single-cell atlases [7,36]. reveal transitional states of **Sertoli** cells through fetal/neonatal stages and at puberty, with gene modules for **FSH** responsiveness, **AR** acquisition, and paracrine cross-talk to germ cells and peritubular myoid cells. These maps help formulate mechanistic models in which **FSH** drives proliferation and secretory programs (AMH, inhibin B) during infancy; only later does AR-dependent maturation permit meiosis. They also raise testable hypotheses: e.g., whether **FSH** exposure during mini-puberty expands the future **SSC** niche and whether **INSL3** dynamics influence adult Leydig-cell ontogeny.

Practical algorithm (0–12 months)

1. **History & exam.** Note prematurity, birth weight/length, family history, genital exam (phallus length, testis position/size).
2. **When to test.** In suspected endocrine etiologies of micropenis/cryptorchidism, assess **LH/FSH, testosterone, INSL3, AMH, inhibin B** between **1–6 months**; preterm infants should be interpreted against **corrected** age.
3. **Interpretation.**
    - **Low LH/FSH/testosterone** with **low AMH/inhibin B** → central hypogonadism with global gonadal under-stimulation likely.
    - **Low testosterone** with **normal AMH/inhibin B** → selective Leydig dysfunction.

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- **Ambiguous genitalia** in 46,XY → prioritize androgen synthesis/action and **LHCGR** pathways (first-trimester issues), not central hypogonadism.

4. **Management.**
    - **No descent by 6 months** → plan hormonal treatment or **orchiopexy** depending on testis position and etiological diagnosis.
    - **CHH** with absent mini-puberty → discuss **physiologic gonadotropin replacement** (LH/FSH or hCG/FSH), delivered in expert centers with careful monitoring.

Future directions

1. **Normative pediatric INSL3/AMH networks.** Larger, multi-ethnic longitudinal cohorts with harmonized assays are needed to define **reference curves** across gestation-corrected ages and to validate **predictive** links to adult fertility.
2. **Randomized trials of mini-puberty replacement.** Existing case series and systematic reviews justify **controlled** trials comparing pump vs injections, dosing schedules, and long-term outcomes (fertility, testicular growth, descent durability).
3. **Single-cell–guided therapeutics.** Targeting Sertoli maturation pathways and SSC niche signals could optimize **timing/sequence** of FSH and LH/hCG in replacement regimens.
4. **Neuroendocrine patterning.** Although beyond the scope of a purely testicular focus, work on GnRH/LH **pulsatility** underscores that **pattern** communicates biology; infant **timing** may have broader developmental consequences worth integrating with andrologic endpoints.

Conclusions

Mini-puberty is a **time-locked**, **physiologically necessary** activation of the HPT axis that calibrates **Leydig** and **Sertoli** compartments, supports penile growth, and creates a last endocrine window for **testicular descent**. It is **not** a miniature puberty: Sertoli cells remain androgen-insensitive, **AMH** stays high, and spermatogenesis awaits AR-dependent maturation at adolescence. Clinically, recognizing the endocrine **signatures** of mini-puberty—particularly in infants with micropenis and/or cryptorchidism—enables early diagnosis of **central hypogonadism** and justifies **physiologic** gonadotropin replacement in specialized settings. Integrating endocrine dynamics with **single-cell** maps and rigorous **biomarker** analytics offers a coherent framework to improve lifelong reproductive health.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

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b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Epigenetic gene regulation in male germ cell development

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Abstract

Male germ cell development unfolds through a sequence of lineage transitions—primordial germ cell (PGC) specification and reprogramming, prospermatogonial (gonocyte) quiescence and conversion to spermatogonial stem cells (SSCs), amplification/differentiation of spermatogonia, and meiosis followed by spermiogenesis—that are choreographed in part by **epigenetic** mechanisms. DNA methylation is globally erased in PGCs and later re-established *de novo* in the fetal/neonatal male germ line; histone post-translational modifications (PTMs) and Polycomb-group (PcG) repressive systems create chromatin environments that both **permit** and **restrict** gene expression programs; the piRNA pathway safeguards genomic integrity by directing transposable-element silencing. Using lessons from our laboratory's work on chromatin-based memory in gametes and early embryos, and integrating recent insights from male germ cell epigenomics, I discuss: (i) how **PcG complexes (PRC1/PRC2)** and their readers/writers organize developmental competence in spermatogonia; (ii) how **CpG-island (CGI)** methylation is protected during oogenesis by **KDM2A/KDM2B** to prevent aberrant maternal hypermethylation that escapes embryonic reprogramming (a paradigm for how epigenetic promoter chromatin states can be inherited); (iii) the emerging view that **H3K27me3/H2AK119ub** landscapes are dynamically regulated in undifferentiated spermatogonia and may be perturbed by intrinsic or environmental stressors resulting in cryptorchidism; and (iv) opportunities to apply single-cell multi-omics to cryptorchid testes to resolve whether a failed developmental transition from **gonocytes to Adark SSC** reflects the acquisition of intrinsic epigenetic roadblocks. I conclude by outlining a framework in which developmental "flexible repression" by Polycomb must be maintained to keep germline genes poised, while inappropriate switching to **DNA-methylated locked repression** at key promoters may derail fate transitions with clinical consequences for fertility.

Key words Epigenetic, germ cell Polycomb group

Résumé

Le développement des cellules germinales mâles progresse par une succession de transitions lignagères—spécification et reprogrammation des cellules germinales primordiales (PGCs), quiescence des prospérmatogonies (gonocytes) et conversion en

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cellules souches spermatogoniales (SSCs), amplification et différenciation des spermatogonies, puis méiose et spermiogenèse—régulées en partie par des mécanismes épigénétiques. La méthylation de l'ADN est globalement effacée dans les PGCs puis rétablie de novo dans la lignée germinale fœtale/néonatale ; les modifications post-traductionnelles des histones et les systèmes répressifs Polycomb façonnent des états chromatiniens permissifs ou restrictifs ; le système piRNA protège l'intégrité génomique par la répression des éléments transposables. À partir de travaux de laboratoire sur la mémoire chromatinienne dans les gamètes et l'embryon précoce, ainsi que des avancées en épigénomique germinale masculine, nous examinons : (i) le rôle des complexes Polycomb (PRC1/PRC2) dans l'établissement de la compétence développementale des spermatogonies ; (ii) la protection de la méthylation des îlots CpG durant l'ovogenèse par KDM2A/B, évitant une hyperméthylation maternelle échappant à la reprogrammation embryonnaire ; (iii) la régulation dynamique des marques H3K27me3/H2AK119ub dans les spermatogonies indifférenciées et leur possible perturbation par des stress intrinsèques ou environnementaux, notamment dans la cryptorchidie ; et (iv) l'intérêt des approches multi-omiques unicellulaires pour déterminer si l'échec de conversion gonocyte → SSC Adark reflète des blocages épigénétiques intrinsèques.

Nous proposons un modèle où la "répression flexible" assurée par Polycomb doit être maintenue pour garder les gènes germinatifs en état de veille, tandis qu'une transition inappropriée vers une répression verrouillée par méthylation de l'ADN peut compromettre les trajectoires cellulaires, avec des conséquences cliniques pour la fertilité.

Mots-clés: Épigénétique, cellule germinale, groupe Polycomb

Conceptual framework: chromatin states that enable or prevent lineage transitions

We examined how transcription factors and chromatin pathways establish, memorize, and transmit gene-regulatory states across cell divisions and between organismal generations, with a focus on mammalian gametes and preimplantation embryos. We uncovered how histone modifiers gate de novo DNA methylation and how Polycomb-mediated repression prevents an inappropriate conversion to hardwired DNA methylation—are highly relevant to male germ cells. In this proceedings contribution I connect these mechanistic principles to spermatogenic development, highlight new evidence on PcG regulation of undifferentiated spermatogonia, and propose how these insights may illuminate cryptorchidism-associated

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defects in the gonocyte-to-SSC transition described clinically and transcriptionally by others [1,2].

Flexible versus locked repression

Chromatin encodes two broad, functionally distinct states of gene silencing.

(i) Polycomb-mediated repression involves **PRC1** (depositing **H2AK119ub**) and **PRC2** (depositing **H3K27me3**), typically at promoters and developmental enhancers. Polycomb repression is *flexible*: it keeps key developmental fate genes off, yet competent to be activated upon receiving appropriate cues [3-7].

(ii) DNA-methylation–mediated repression at **CGI promoters** is *locked*: once promoters become CpG-methylated by **DNMT3A/DNMT3L**, accessibility and TF binding are strongly curtailed, and re-activation is difficult [8–10]. Our recent work demonstrates how these layers are mechanistically **interlinked**—and how perturbations move genes from a flexible (Polycomb) to a locked (DNA-methylated) state with heritable consequences [8,9].

Intergenerational chromatin memory

A central lesson from our lab is that choices made in the female **germ line** can be transmitted to the **embryo**. In mouse oocytes, the demethylases **KDM2A/KDM2B** restrain **H3K36me2** at CGIs, preventing **DNMT3A** from methylating promoters. If KDM2A/KDM2B proteins are absent, aberrant hypermethylation accumulates at CGI promoters in growing oocytes, persists into **four-cell embryos**, and **represses** the maternal allele of key developmental genes. Strikingly, removal of **DNMT3A** in the oocyte rescues the preimplantation lethality of *Kdm2a*/*Kdm2b* deficient embryos, proving causality [8]. Although discovered in oocytes, the principle—that the **histone mark balance at promoters gates access of DNMT3A**, determining whether repression stays Polycomb-flexible or becomes DNA-methylation-locked—applies to **spermatogonia** as well [10].

A timeline of male germ cell epigenetics

PGC erasure and male-specific *de novo* methylation

PGCs undergo global **DNA demethylation** (active and passive) during migration and gonadal colonization, removing parental methylation genome-wide including at CGIs, imprinting control regions and many endogenous repetitive viral elements. In the male germline, prospermatogonia acquire high levels of ***de novo*** methylation during late fetal/neonatal stages under the control of *de novo* DNA methyltransferases **DNMT3A, DNMT3C** and **DNMT3L**. This re-methylation licenses future spermatogenesis and transposon restraint, in part in cooperation with the **piRNA** pathway (MILI, MIWI2 and cofactors) [11-14].

Spermatogonial chromatin and PcG landscapes

Undifferentiated spermatogonia (including SSCs) exhibit PcG-enriched chromatin: **PRC1/PRC2** partition developmental regulators into a repressed-but-poised compartment characterized by **H3K27me3** and **H2AK119ub**. During commitment to differentiation, **global**

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H3K27me3 levels decrease and specific PcG domains are remodeled, allowing lineage genes to be activated with proper timing [3-6]. In mouse, PRC1 constrains premature activation of germline genes and coordinates their **timely** expression during spermatogenesis; PRC1 loss disrupts this choreography [3-4]. In adult mice, PRC1 shields undifferentiated spermatogonia from stochastic differentiation, helps maintain slow cycling, and directs orderly commitment; **PRC2-H3K27me3** emerges as an epigenetic hallmark of this population [3-5].

Meiosis, spermiogenesis, and paternal chromatin

Entering meiosis entails broad transcriptional reprogramming with stage-specific histone marks (e.g., **H3K4me3**, **H3K9me3**, **H3K27me3** dynamics) and recruitment of germline TFs. Post-meiotically, massive histone eviction and **protamine** incorporation fully reprograms the paternal epigenome. Nonetheless, few **nucleosomes** are retained in sperm, the function of which remains to be identified [15-19]. PcG factors (e.g., **SCML2**) and modulators such as **EZHIP** can tune PRC2 activity in gonads, with consequences for H3K27me3 abundance during spermatogenesis [20].

Polycomb systems in spermatogonia: poised genomes and developmental timing

PRC1/PRC2 logic

In mESCs and in vivo, **H2AK119ub** (PRC1) often shows rapid restoration after DNA replication, whereas **H3K27me3** (PRC2) accumulates more slowly, suggesting that PRC1 can **prime** domains for PRC2 spreading [21]. In male germ cells, convergent evidence indicates that **PRC1 establishes and maintains** repressive compartments in undifferentiated spermatogonia, preventing inappropriate transcription and preserving stemness; during differentiation, selected PcG domains are dismantled so germline programs can proceed [2-5,10].

Consequences of PcG imbalance

If PcG repression is weakened, developmental and pro-apoptotic genes become ectopically activated [6]. If repression is inappropriately hardened by promoter DNA methylation, a different pathology arises—failure to activate required genes when differentiation cues arrive [9]. Either imbalance may also prevent the **gonocyte to Adark SSC** transition observed in cryptorchid testes, where failure to establish the Adark reserve population presages subfertility [1]. A crucial inference is that **maintaining Polycomb-facilitated flexibility**, not converting promoters into DNA-methylated hard-locks, might be critical in this window.

Temperature stress and cryptorchidism: PcG marks under pressure

A surgically induced cryptorchid model has recently been established in adult mice to profile the epigenetic landscape of **undifferentiated spermatogonia**. The key observation is a **local**

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(not global) loss of **H3K27me3** and **H3K9me3** at PcG-regulated genes, accompanied by **upregulation** of developmental and pro-apoptotic pathways. Cryptorchid spermatogonia show increased expression of **H3K27 demethylases KDM6A/KDM6B**, and elevated temperature directly induces Kdm6a/Kdm6b in germline stem cell culture [6]. These data dovetail with earlier work linking abnormal H3K27me3 to impaired SSC maintenance and differentiation [3]. They point to a plausible **mechanism** whereby aberrant scrotal heat exposure—central to cryptorchidism—**erodes** PcG-mediated flexible repression, de-repressing stress-response and developmental genes, precipitating either apoptosis or mis-timed differentiation. Whether a similar chromatin response is observed in developmentally cryptorchid juvenile mice, and whether subsets of promoters may subsequently convert to **DNA-methylated** locked states remains to be tested in longitudinal models.

Promoter choice: preventing unwanted DNA methylation at CGIs

Lessons from the oocyte

Our 2025 studies establish that the **KDM2A/KDM2B** histone demethylases, binding to unmethylated CpG islands, **remove H3K36me2** and thereby limit **DNMT3A** access to promoter CGIs in **growing oocytes**. In their absence, widespread **CGI hypermethylation** arises which is **inherited** to four-cell embryos, where it leads to **allele-specifically** transcriptional repression from the maternal genome. Deleting ***Dnmt3a*** in oocytes rescues embryonic lethality, proving that aberrant DNA methylation is the causal lesion [9]. Mechanistically, we further uncover a promoter "grammar" in which the **antagonism** between H3K4 methylation (protective) and H3K36 methylation (permissive for DNMT3A) and local sequence features determines whether a promoter flips from Polycomb to DNA methylation when KDM2A/KDM2B protection is removed [9].

Extrapolation to male germ cells

While male prospermatogonia do undergo **physiologic** *de novo* methylation, the hypothesis is that many **stem/progenitor promoters** in undifferentiated spermatogonia must remain **unmethylated** and **Polycomb-repressed** to preserve flexibility [5,10]. Inappropriate promoter methylation—due to chronic stress, imbalance of chromatin modifying enzymes (e.g., altered **KDM2** family function, excessive **H3K36** signals), or mis-coupling of PRC1/PRC2—could permanently lock out essential fate genes [10]. Single-cell bisulfite DNA methylation and chromatin modification profiling via CUT&TAG/CUT&RUN studies in human cryptorchid testes could test whether high-risk infants show aberrant CGI hypermethylation (locked repression) at **Adark-associated** gene sets.

Transposon control and the piRNA pathway: a parallel layer

Retrotransposons (LINE-1, IAP, etc.) are silenced in male germ cells via **piRNA-directed** DNA methylation and repressive histone marks. Failure of the **MILI/MIWI2** axis compromises

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genome integrity and induces spermatogenic arrest. Notably, Polycomb and piRNA systems intersect functionally: PcG repression can help **buffer** developmental genes while piRNA machinery targets repeats, collectively allowing germ cells to proceed through meiosis without genotoxic stress [5,10-13]. Temperature stress may also perturb piRNA biogenesis; integrating **small RNA** profiling with PcG mapping in cryptorchid models should reveal whether TE de-repression contributes to the observed SSC attrition.

From gonocyte to Adark SSC: a testable epigenetic model

Clinical and histological studies indicate that cryptorchidism with high risk for adult infertility is characterized by the **absence of Adark spermatogonia**—the reserve SSC pool in infant boys. Expression profiling of infant testes stratified by Adark status shows widespread **low expression** of genes implicated in SSC identity and differentiation, consistent with a **blocked transition** from **gonocyte (prospermatogonia)** to **SSC** [1,2]. Re-interpreting such signatures through an epigenomic lens suggests two, not mutually exclusive, failure modes:

1. **Promoter lock model**: Impaired developmental progression of gonocytes, intrinsic or due to exogenous chronic stress, may alter promoter chromatin from **Polycomb** to **DNMT3A-mediated** DNA methylation at a subset of **SSC competence genes**, irreversibly preventing their activation when hormonal cues arrive (by analogy to the **KDM2A/KDM2B** paradigm in oocytes and KDM2A function in spermatogonia) [9.10,].
2. **PcG erosion model**: Heat stress and/or inflammatory cue may increase **KDM6A/KDM6B** activity, **locally** eroding **H3K27me3**, and de-repressing developmental/pro-apoptotic modules prematurely, exhausting or eliminating the SSC progenitor pool [3.5,6].

These models make distinct predictions. While the PcG erosion model yields **loss of H3K27me3** (without DNA methylation change) at specific loci, the lock model yields **new promoter CGI methylation**. Single-cell multi-omics on pediatric cryptorchid testes can discriminate them.

Single-cell multi-omics: resolving cell-intrinsic versus niche effects

The testis is a mosaic of germ and somatic cells. Bulk profiling can be misleading. Single-cell atlases in the human testis have already delineated **SSC sub-states** and somatic niches. Extending them with **single-cell CUT&TAG** (H3K27me3/H2AK119ub), **single-cell methylome**, and **small-RNA** sequencing in cryptorchid and control testes (correcting for age, temperature exposure, and surgery timing) will identify **cell-intrinsic** chromatin lesions in germ cells versus **extrinsic** niche signals (Sertoli, Leydig, peritubular myoid). A prospective study that pairs **longitudinal** sampling with **orchiopexy timing** could test whether early surgery prevents PcG erosion and reduces promoter locking at SSC-critical genes.

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Translational implications

1. **Biomarkers.** PcG state reporters (e.g., **H3K27me3** at selective gene promoters) in **germ cell-derived chromatin fragments** or in **testicular biopsy nuclei** could serve as biomarkers for SSC reserve integrity.
2. **Therapeutic window.** If cryptorchidism elevates **KDM6A/KDM6B** activity and depletes **H3K27me3**, small-molecule **inhibitors for KDM6** (already explored in oncology) might, in principle, stabilize PcG repression—though safety, specificity, and ethics in infants are major hurdles.
3. **Avoiding promoter lock.** Understanding the **KDM2 – H3K36 methylation – DNMT3A** axis in male germ cells may point to strategies that prevent **inappropriate CGI methylation** in SSCs, preserving flexibility for later activation.
4. **Counseling.** Mechanistic knowledge strengthens the rationale for **early orchiopexy** (temperature normalization) and underscores why **delays** may have irreversible epigenetic costs.

Outlook: rules that generalize across gametes

Our oocyte studies show that "flexible" Polycomb repression can be converted to "rigid" DNA-methylation repression at promoters if **KDM2A/KDM2B**-mediated protection is lost; this locked repression is **heritable** into the embryo and **causally** impairs development [9]. In male germ cells, we posit that *the same decision logic applies* to promoters of **SSC competence** and **differentiation** genes. Meanwhile, **PRC1/PRC2** keep undifferentiated spermatogonia poised; when disrupted (by genetics or environment), timing and survival are perturbed [3-6]. Finally, **modulators** such as **EZHIP** can dampen PRC2 activity in the gonads, reminding us that PcG dosage is tightly tuned in the germ line [20]. Bringing these strands together, a unifying epigenetic model of male germ cell development emerges: Polycomb builds the poised male germ line landscape; KDM2A/KDM2B-guarded CGIs keep promoters unmethylated; DNMT3A writes stable locks where appropriate (endogenous repetitive elements, imprints but not at SSC-critical promoters); piRNA system patrols repeats; and temperature or inflammation stress can upset the balance. [21].

Materials and Methods (conceptual framework)

This proceedings' contribution synthesizes published primary data and reviews, including mechanistic experiments from our laboratory and others. Where appropriate, I refer to **mouse** models (oocyte *Kdm2a*/*Kdm2b*, *Dnmt3a*; spermatogonial PcG perturbations; cryptorchid surgery) and to human single-cell atlases and clinical observations. No new datasets are presented here; rather, I propose **testable hypotheses** and **study designs** (single-cell multi-omics of cryptorchid testes) grounded in the cited literature.

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Conclusions

Male germ cell development depends on a choreography of chromatin states: **Polycomb** maintains a poised genome in undifferentiated spermatogonia; **piRNA** and **DNA methylation** secure the genome against transposons; and **promoter CGI protection** prevents conversion to irreversible repression. The recent demonstration that **promoter choice** in the oocyte can be **memorized** into the embryo provides a strong precedent for similar modes of regulation in the male germ line. In cryptorchidism, temperature-induced **PcG erosion** and potential promoter **locking** offer mechanistic explanations for the failure of the **gonocyte to Adark** transition. The immediate priority is to **map** PcG marks, promoter methylation, and small RNAs at **single-cell resolution** in infant testes at risk. The medium-term goal is to determine whether restoring the **poised** state can preserve future fertility.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Impact of disturbed piRNA biogenesis on transposon de-repression and male infertility

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Abstract

Defects in germline genome integrity and gene regulation frequently underpin male infertility. The PIWI–piRNA pathway safeguards the male germline against transposable elements (TEs) from fetal germ cells through the process of spermatogenesis. Over the past decade, particularly in recent years, exome and genome sequencing of large cohorts with clinically well-defined phenotypes has revealed that monogenic causes essentially contribute to the disease. Using data from the Münster-based MERGE cohort, we have shown that inherited defects in human piRNA biogenesis are also an important and actionable cause of spermatogenic failure. Accordingly, we have identified biallelic variants across core and accessory piRNA factors, established genotype–phenotype correlations, demonstrated loss of pachytene piRNAs in patient testis, and linked specific gene variants to transposon de-repression in human spermatogonia. Here, I summarise these recent findings and discuss the differences in the reproductive phenotypes between human variant carriers and knockout models of the mice orthologues.

Key words Infertility, Transposon, piRNA

Résumé

Les défauts d'intégrité génomique et de régulation génique de la lignée germinale constituent des causes majeures d'infertilité masculine. La voie PIWI–piRNA protège les cellules

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germinales masculines, depuis le stade fœtal jusqu'à la spermatogenèse, contre les éléments transposables (TEs). Au cours de la dernière décennie, et tout particulièrement ces dernières années, le séquençage d'exomes et de génomes dans de larges cohortes phénotypiquement bien caractérisées a mis en évidence une contribution substantielle de causes monogéniques.

À partir de la cohorte MERGE (Münster), nous avons montré que les anomalies héréditaires de la biogenèse des piARN humains constituent une cause importante et actionnable d'insuffisance spermatogénétique. Nous avons identifié des variants bialléliques affectant des facteurs centraux ou accessoires de la voie piRNA, établi des corrélations génotype–phénotype, démontré la perte des piARN pachytènes dans les testicules de patients et relié certains variants géniques à une dérégulation des transposons dans les spermatogonies humaines. Cette synthèse présente ces avancées récentes et discute les divergences phénotypiques entre porteurs humains et modèles murins invalidés pour les orthologues correspondants.

Mots-clés: Infertilité, transposon, piRNA

Introduction

Semen analysis remains the starting point for male infertility diagnostics, and a substantial fraction of patients present with reduced sperm counts up to complete azoospermia, often reflecting intrinsic spermatogenic failure rather than obstructive causes. Historically, genetic testing focused on chromosomal aberrations (e.g., Klinefelter syndrome) and AZF microdeletions on the Y chromosome. Apart from *CFTR* for obstructive azoospermia, sequencing of single disease genes to identify monogenic causes remains underdetermined. However, more than three-quarters of human genes are expressed in testis, thousands are testis-enriched, and hundreds are testis-specific, implying that monogenic aetiologies could be expected. Indeed, mouse knockout studies have linked many genes to spermatogenic arrest in mice. However, the extent to which pathogenic genetic variants in human genes contribute to the disease remains unexplored. Recognising this, we established the MERGE cohort, comprising genome and exome sequencing data from approximately 2,800 infertile men, mainly affected by reduced sperm count to systematically uncover monogenic causes of male infertility and to translate valid disease genes into diagnostic practice [1,2]. By this approach, we increased the diagnostic yield in patients with azoospermia by 7%. Nevertheless, further research is required to expand the spectrum of valid disease genes.

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Landscape of human genetic variants contributing to disturbed piRNA pathway

To identify the key biological pathways that contribute to male infertility, we performed a Gene Ontology (GO) analysis on a list of genes in which homozygous loss-of-function variants were identified in the MERGE cohort. This revealed that piRNA processing is a key biological process contributing to the disease [3]. piRNAs represent the most abundant subgroup of regulatory small non-coding RNAs in the testis. They bind to PIWI proteins, a subclade of argonaute proteins, and guide them to silence transposable elements (TEs), mobile DNA sequences that are capable of copying and inserting themselves throughout the genome. Impaired piRNA activity can cause uncontrolled TE activity and threaten genome integrity. In mice, mutations in genes essential for piRNA biogenesis, such as *Piwil1*, *Tdrd1*, *Mael*, or *Mili*, lead to meiotic failure, and infertility [4]. In humans, two key genes of the piRNA pathway, *PNLDC1* and *FKBP6*, had been linked to biallelic pathogenic variants in the piRNA pathway [5,6]. However, a comprehensive analysis of the contribution of a disturbed piRNA pathway to human male infertility has been lacking.

In 2024, our study on more than 2400 MERGE samples identified 31 infertile men with biallelic high-impact variants in 14 piRNA-pathway genes**,** including *PIWIL1*, *GTSF1*, *TDRD1*, *TDRD12*, *GPAT2***,** *MAEL, and TDRD9* [3]. Of these patients, 12 were carriers of biallelic loss-of-function variants, reflecting the phenotype of a human 'knockout'. The variant carriers were affected by spermatogenic failure, with a significant proportion exhibiting azoospermia or cryptozoospermia. The testicular morphology of the variant carriers confirmed impaired spermatogenesis, displaying a broad spectrum of phenotypes ranging from hypospermatogenesis to a Sertoli cell-only type of histology. However, with respect to a single gene, the testicular phenotypes were mainly concordant. A gene-by-gene comparison of the testicular phenotypes in human variant carriers and knockout mice showed several gene-specific differences. For some genes, e.g. *PIWIL2*, the spermatogenic arrest occurred at an earlier stage in humans than in mice. For others, e.g. *TDRD9,* spermatogenesis progressed to later stages than those observed in the respective mouse knockout model. We demonstrated the absence of the affected protein in the testicular tissue of several homozygous stop-gain or frameshift variant carriers, proving the expected loss of protein function. Small-RNA sequencing of RNA samples derived from testicular biopsies of selected variant carriers resulted in reduced levels of pachytene piRNAs, thereby linking the genetic variations to

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disturbed piRNA biogenesis. Immunohistochemical staining for the LINE1 marker protein LINE1 ORF1p revealed upregulation of the protein in spermatogonia in several human variant carriers, including patients affected by biallelic variants in *TDRD12*, *GPAT2* and *FKBBP6*. This LINE1 upregulation serves as a molecular signature of transposon de-silencing due to disrupted piRNA function. These findings redefine a subset of idiopathic male infertility as a distinct molecular entity: piRNA pathway deficiency–associated spermatogenic failure. Clinically, this work expands the catalogue of diagnostic targets for genetic testing panels.

Practical recommendations for clinicians and laboratories

1. Men with non-obstructive azoospermia (NOA) or severe oligozoospermia for which there are no obvious systemic or iatrogenic causes should receive comprehensive genetic analyses, including screening for chromosomal aberrations and microdeletions on the Y chromosome, as well as exome/genome sequencing of a curated list of disease genes gene with sufficient clinical validity.
2. Data on the chances of testicular sperm extraction for specific genes, health comorbidities, and the outcome of artificial reproductive techniques should be collected to improve future patient care and counselling.

Conclusions

This study provides definitive evidence that inherited defects in piRNA biogenesis genes are a direct cause of human male infertility. It establishes that the mechanisms underlying germline genome integrity, which were previously characterized mainly in model organisms, are conserved in humans. However, it also highlights that phenotypic findings from mice cannot be directly extrapolated to humans in the case of piRNAs. Further identification and functional characterisation of additional variants is required in order to systematically determine the reproductive phenotypes associated with impaired piRNA pathway function in humans.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

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d) Competing interests Author/s declare that they have no competing interests

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Biological effects on early-stage spermatogenesis in cryptorchidism

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Abstract

Cryptorchidism disrupts the earliest transitions of male germ cells and compromises long-term fertility potential. Here I summarize our basic and clinical investigations into (i) germ-celland Sertoli-cell changes in experimental and human cryptorchidism, (ii) molecular candidates involved in the differentiation process from gonocytes to spermatogonia, (iii) the timing benefit of early orchiopexy, and (iv) endocrine modifiers, including androgen signaling and neonatal thyroid status. Across rat models and pediatric cohorts, we observe delayed gonocyte resettlement to the basement membrane, reduced undifferentiated type-A spermatogonia, Sertoli-cell junctional disorganization (Claudin-11/blood-testis barrier (BTB), and transcriptional shifts implicating histone demethylation (KDM5A), FOXO1 regulation by miR-135a, and tight junction remodeling. In bilateral cryptorchidism, lower inhibin-B–to-FSH ratios track with depressed germ-cell counts at biopsy; combining AMH, inhibin B, and FSH yields strong ROC performance for predicting poor germ-cell indices, supporting pre-operative endocrine triage.

Key words Cryptorchidism, hypothyroidism, Sertoli cell, germ cell

Résumé

La cryptorchidie perturbe les premières transitions des cellules germinales mâles et compromet le potentiel de fertilité à long terme. Cette synthèse présente nos travaux fondamentaux et cliniques portant sur : (i) les altérations des cellules germinales et de Sertoli dans la cryptorchidie expérimentale et humaine ; (ii) les candidats moléculaires impliqués dans la différenciation gonocyte → spermatogonie ; (iii) le bénéfice temporel d'une orchidopexie précoce ; et (iv) les modulateurs endocriniens, incluant la signalisation androgénique et le statut thyroïdien néonatal.

Dans les modèles murins et les cohortes pédiatriques, nous observons un retard du repositionnement des gonocytes vers la membrane basale, une diminution des spermatogonies A indifférenciées, une désorganisation jonctionnelle des cellules de Sertoli (Claudin-11/barrière hématotesticulaire), et des remaniements transcriptionnels impliquant la déméthylation histonique (KDM5A), la régulation de FOXO1 par miR-135a et le remodelage

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des jonctions serrées. En cryptorchidie bilatérale, le ratio inhibine B/FSH reflète la pauvreté germinale à la biopsie, et l'association AMH–inhibine B–FSH améliore la prédiction des faibles indices germinaux.

Mots-clés: Cryptorchidie, hypothyroïdie, cellule de Sertoli, cellule germinale

Introduction

Cryptorchidism is among the most common congenital anomalies in boys and is a leading risk factor for subfertility and testicular cancer later in life. Neonatal, transient hypothyroidism increases undifferentiated spermatogonia in rats and associates with higher

germ-cell counts in boys with a history of congenital hypothyroidism, suggesting a modulatory role for thyroid hormone in early spermatogonial differentiation. Collectively, these data argue that cryptorchidism perturbs germ-cell fate decisions and Sertoli barrier maturation during a narrow postnatal window—defects that are partly mitigated by timely orchiopexy and potentially readable by minimally invasive hormone panels. [1–3] The two-phase model of testicular descent—an insulin-like 3 (INSL3)-dominated transabdominal phase and an androgen-dependent inguinoscrotal phase—provides a useful framework for mechanistic thinking, and both phases can be perturbed by environmental anti-androgens in experimental systems [2–4]. Beyond mechanical malposition and thermal stress, our work focuses on germ-cell biology during the first postnatal months ("mini-puberty") and prepuberty, when gonocytes should migrate to the basement membrane, reprogram into undifferentiated spermatogonia (including UTF1+ and GFRA1+ subsets), and establish the spermatogonial stem-cell (SSC) pool that seeds lifelong spermatogenesis [1,2,4]. We summarize evidence from our laboratory and university hospital in Nagoya, integrating transcriptomic screens, animal models, and pediatric biopsy-linked endocrine phenotypes.

Methods overview (selected studies)

Experimental cryptorchidism and anti-androgen exposure

We established rat cryptorchidism by fetal exposure to the androgen receptor antagonist flutamide during gestational days 14–20 (androgen-dependent window), producing high rates of undescended testes with impaired spermatogenesis at puberty [2,4] Histology, immunohistochemistry (IHC), and electron microscopy were used to quantify seminiferous tubule diameter, germ-cell apoptosis (TUNEL), and Sertoli tight junctions (claudin-11).

Timing of orchiopexy

In flutamide-exposed rats, we performed orchiopexy at 4–7 weeks and assessed testis architecture at 10 weeks, comparing with non-operated cryptorchid controls.

Germ-cell lineage readouts

We applied IHC for UTF1 (gonocyte/undifferentiated spermatogonia marker), DDx4/MVH (germ-cell cytoplasmic marker), and GFRA1 (undifferentiated type-A spermatogonia/SSC compartment) with time-course quantification at postnatal day (PND) 9 and beyond [5,11].

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Microarray profiling at PND9 contrasted descended vs undescended testes; separate microRNA arrays surveyed regulatory small RNAs [12,13].

Sertoli-cell barrier analysis

Claudin-11 distribution (BTB component) was evaluated by confocal microscopy and ultrastructure by electron microscopy; junctional integrity was scored per tubule cross-section [6,14].

Human biopsy-linked studies and endocrine profiling

In a single-center pediatric series of orchiopexy (N=323; biopsies analyzed in N=146), we quantified germ-cell number per tubule cross-section (G/T; DDx4 based), testicular volume, and recorded laterality/position. Pre-operative serum inhibin B, AMH, LH, FSH were analyzed; ratios (e.g., inhibin B:FSH) and receiver–operating characteristic (ROC) analyses were used to predict low G/T [15].

Thyroid hormone perturbation

To model neonatal hypothyroidism, dams received propylthiouracil in drinking water for 7 days postpartum; male offspring testes were examined for GFRA1+ and DDx4+ cells. In our human series, we compared germ-cell indices in cryptorchid boys with vs without a history of congenital hypothyroidism [16].

Results

Anti-androgen fetal exposure produces cryptorchidism with early germ-cell and Sertoli defects

Fetal flutamide exposure yielded consistent cryptorchidism and pubertal spermatogenic impairment regardless of dose. Early postnatal testes revealed (i) reduced seminiferous tubule diameter, (ii) increased apoptosis [10], and (iii) disorganized tight-junctional architecture with fragmented claudin-11 patterns compared with linear BTB belts in controls [14]. These features align with broader evidence that androgens organize the inguinoscrotal phase and that anti-androgen exposure deranges descent and subsequent testis maturation [1–3].

Early orchiopexy partially rescues architecture and germ-cell indices

When orchiopexy was performed at 4–7 weeks in the rat model, testes harvested at 10 weeks displayed improved tubule caliber and reduced histological damage compared with uncorrected cryptorchid testes; the benefit diminished as age at correction increased, underscoring a narrow therapeutic window [17].

Delayed gonocyte resettlement and reduced SSC establishment

At PND9, undescended testes contained more centrally located gonocytes and fewer peripherally positioned type-A spermatogonia than controls, consistent with delayed migration/resettlement to the basement membrane (UTF1+ quantification) [5,11]. This spatial lag implies SSC pool under-establishment, a likely substrate for later quantitative germ-cell loss.

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Transcriptomic candidates: chromatin remodeling and FOXO1 regulation

Microarray at PND9 identified upregulation of Kdm5a (H3K4 demethylase) in undescended testes; IHC localized KDM5A to germ cells . Given KDM5A's role in removing H3K4 methylation (an activation-associated mark), over-activity could blunt transcriptional programs needed for SSC differentiation, converging with independent evidence that histone modifications gate SSC fate [7,12]. MicroRNA analysis showed downregulation of miR-135a in undescended testes, and functional assays supported miR-135a targeting of FOXO1; nuclear FOXO1-positive SSCs were reduced in cryptorchid testes [7]. Since FOXO1 is pivotal for SSC maintenance and stress responses, impaired miR-135a–FOXO1 tuning offers a plausible axis for early germ-cell vulnerability [7,13].

Sertoli-cell barrier maturation is delayed and mispatterned

Claudin-11 staining, normally forming continuous linear belts at puberty, appeared discontinuous/patchy in cryptorchid testes up to 4 weeks; the proportion of tubules with abnormal claudin-11 distribution was significantly higher than in controls. Ultrastructural analysis corroborated BTB disarray, and apoptosis was increased, consistent with the notion that premature BTB failure deprives developing germ cells of niche protection [6,14].

Human cryptorchid testis: shared transcriptional signatures and germ-cell depletion

Subtractive cDNA analysis comparing biopsies from hydrocele vs cryptorchid boys identified 18 up-regulated and 16 down-regulated genes in cryptorchid testes; qPCR confirmed increased TPT1, EEF1A1, and NuMA1 gene, each expressed in germ cells across spermatogenesis [18]. Morphometry showed reduced G/T in bilateral vs unilateral cryptorchidism.

Endocrine correlates and prediction of germ-cell loss

In the pediatric cohort, bilateral cryptorchidism showed significantly lower inhibin B:FSH ratios and lower G/T than unilateral cases. In bilateral cases <24 months, composite endocrine indices (inhibin B:FSH, AMH:FSH, and FSH) predicted low G/T with high accuracy (AUC >0.9), enabling cutoffs to triage boys at highest risk for germ-cell loss before surgery. These observations mirror broader literature linking inhibin B (Sertoli output) and FSH (pituitary input) with Sertoli/germ-cell status in early life [2,15].

Neonatal hypothyroidism increases undifferentiated spermatogonia

In the transient neonatal hypothyroid rat model,PND7 and PND20 males males had higher counts of DDx4+ germ cells and GFRA1+ undifferentiated spermatogonia than controls, implying that reduced neonatal thyroid hormone favors SSC-state retention or delayed differentiation [9]. In boys with cryptorchidism, a history of congenital hypothyroidism associated with higher germ-cell numbers at biopsy, paralleling the animal data. These preliminary findings suggest that thyroid hormone participates in in the differentiation process from gonocytes to spermatogonia. [16]

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Ancillary lines of inquiry

Testicular DSD and SOX3 dosage as a model of testis determination

In SRY-negative 46,XX testicular DSD, we identified upregulation of ROCK1 in XX testis biopsy material [19] and, in a series of four cases, recurrent copy-number gain upstream of **SOX3** on chromosome X; given structural similarity between SOX3 and SRY, these data support an SRY-independent SOX3 pathway for human testis determination in a subset of cases [10]. SOX3 duplications have been reported by others in SRY-negative 46,XX testicular DSD [10,20].

Glucose metabolism and PGC/gonocyte programming

In embryoid-body systems directing mouse pluripotent cells toward germ-cell–like lineages, higher glucose media increased MVH/DDx4-positive germ-cell-like cells; subtraction cloning identified Txnip, RuvBl2, Pttg, and and other candidates enriched under high glucose, with expression confirmed in fetal through adult germ cells. Although mechanistic dissection remains pending, the data point to metabolic gating of germ-cell fate acquisition [21].

Discussion

Our integrated datasets converge on a simple narrative: cryptorchidism perturbs two coupled developmental programs during a restricted postnatal window—(i) germ-cell lineage timing (basement-membrane resettlement and SSC establishment) and (ii) Sertoli-cell barrier maturation. Fetal anti-androgen exposure reproduces both positional failure and early testicular immaturity; early orchiopexy partially rescues the niche, strengthening the case for prompt surgery [2,3,10,11,14,17].

Germ-cell timing defects. The accumulation of centrally located gonocytes at PND9 in undescended testes implies a failure to complete the migration and differentiation sequence that should yield UTF1+/GFRA1+ SSCs at the periphery. Chromatin remodeling (KDM5A upregulation; decreased H3K4 methylation) and FOXO1 dysregulation via reduced miR-135a provide mechanistic footholds that could slow exit from a progenitor state or impair SSC maintenance [7,8]. These axes align with mounting evidence that histone and transcription-factor circuits set SSC competence and stress resilience [12,13].

Sertoli barrier and microenvironment. BTB formation is a hallmark of pubertal Sertoli maturation; disorganized claudin-11 belts and ultrastructural BTB defects in cryptorchid testes match reports that BTB integrity is crucial to germ-cell survival and progression [6]. A compromised BTB likely amplifies thermal and oxidative stress sensitivity and facilitates apoptosis, compounding primary germ-cell timing defects [14].

Clinical translation. Our endocrine–histology correlations suggest that low inhibin B:FSH (and related AMH indices) can flag bilateral cryptorchid boys at high risk of germ-cell depletion before orchiopexy. Such panels could prioritize earlier intervention and inform parental counseling. The strong ROC values in bilateral cases underscore the value of combining Sertoli-derived (inhibin B, AMH) and pituitary (FSH) readouts [15].

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Endocrine modulators beyond androgens. The neonatal hypothyroidism data in rats and the signal in boys with congenital hypothyroidism are provocative: thyroid hormone appears to influence the tempo of SSC emergence vs maintenance. While the directionality (more GFRA1+ cells) could be protective or reflect delayed differentiation, either scenario has implications for the timing of repair and adjuvant therapies [9,16].

Limitations and next steps. Many results are single-center and require replication across populations. Microarray signals (KDM5A/miR-135a) need causal testing in vivo (gain-/loss-of-function in SSCs) and integration with single-cell multi-omics across cryptorchid and control testes. BTB alterations should be mapped against functional paracellular permeability assays. Endocrine cutoffs demand external validation and age-adjusted nomograms.

Conclusions

Early-stage spermatogenesis in cryptorchidism is derailed by delayed gonocyte migration/differentiation and immature Sertoli-cell barrier assembly, processes that are transcriptionally and epigenetically tunable and endocrinologically readable. Early orchiopexy improves architecture, and composite Sertoli/pituitary hormone panels can help identify boys at greatest risk of germ-cell loss. The interplay of androgen, thyroid, and chromatin-regulatory pathways defines an actionable window for preserving the SSC pool and lifelong fertility potential. [2,3,6–9]

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Puberty in boys with a history of cryptorchidism

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Abstract

Cryptorchidism—the failure of one or both testes to descend into the scrotum—is the most common genital anomaly in male infants and a salient risk factor for hypogonadism, impaired spermatogenesis, and testicular malignancy later in life. The neonatal hypothalamic–pituitary–testicular (HPT) axis surge ("mini-puberty") provides a natural stress test of the infant testis, and accumulating prospective evidence shows that boys with cryptorchidism display biochemical signatures consistent with suboptimal Sertoli-cell function already in early infancy. Puberty, however, is orchestrated by a renewed activation of the HPT axis and a rapid expansion of seminiferous tubule mass; how these processes unfold in boys with a history of cryptorchidism is clinically decisive for lifetime reproductive potential. Drawing on Nordic birth cohorts from Denmark and Finland, longitudinal adolescent follow-ups, and contemporary guideline-based care, this paper synthesizes the physiology and clinical course of puberty after cryptorchidism. We summarize (i) geographic variation in cryptorchidism and the "testicular dysgenesis" paradigm; (ii) infant endocrine phenotypes that stratify later risk; (iii) trajectories of pubertal onset and tempo; (iv) testicular growth and hormone dynamics (inhibin B, AMH, FSH, LH, testosterone); (v) modifying effects of laterality, position at diagnosis, spontaneous descent versus orchiopexy, and timing of surgery; and (vi) clinical implications for counselling and follow-up. The weight of evidence indicates that pubertal timing per se is typically normal in prior cryptorchid boys, but testicular growth during puberty is blunted—most clearly in bilateral and formerly non-palpable/abdominal testes—and accompanied by lower inhibin B and modestly higher FSH (with broadly similar serum testosterone), signaling reduced Sertoli-cell mass and germ-cell output. Early orchiopexy (by 6–12 months) and spontaneous descent are associated with more favorable adolescent testicular volume and endocrine profiles than late correction. These insights refine risk stratification and motivate structured, biomarker-informed pubertal surveillance to optimize fertility counselling.

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Key words: cryptorchidism, mini puberty, Sertoli cell, treatment

Résumé

La cryptorchidie – absence de descente d'un ou des deux testicules dans le scrotum – est l'anomalie génitale la plus fréquente chez le nourrisson de sexe masculin et un facteur de risque majeur d'hypogonadisme, d'altération de la spermatogenèse et de cancer testiculaire à l'âge adulte. La poussée néonatale de l'axe hypothalamo-hypophyso-testiculaire (HPT), surnommée « mini-puberté », constitue un test de stress naturel pour le testicule du nourrisson. Des données prospectives de plus en plus nombreuses montrent que les garçons atteints de cryptorchidie présentent, dès la petite enfance, des signatures biochimiques compatibles avec un fonctionnement suboptimal des cellules de Sertoli. La puberté, quant à elle, est orchestrée par une nouvelle activation de l'axe HPT et une expansion rapide de la masse des tubes séminifères. Le déroulement de ces processus chez les garçons ayant des antécédents de cryptorchidie est déterminant pour leur potentiel reproductif tout au long de leur vie. S'appuyant sur des cohortes de naissance Nordiques (Danemark et Finlande), des suivis longitudinaux à l'adolescence et les recommandations actuelles en matière de soins, cet article synthétise la physiologie et l'évolution clinique de la puberté après une cryptorchidie. Nous résumons (i) la variation géographique de la cryptorchidie et le paradigme de la « dysgénésie testiculaire » ; (ii) les phénotypes endocriniens infantiles qui stratifient le risque ultérieur ; (iii) les trajectoires d'apparition et de rythme de la puberté ; (iv) la croissance testiculaire et la dynamique hormonale (v) les effets modificateurs de la latéralité, de la position au moment du diagnostic, de la descente spontanée versus l'orchidopexie et du moment de l'intervention ; et (vi) les implications cliniques pour le conseil et le suivi. L'ensemble des données probantes indique que le calendrier pubertaire en lui-même est généralement normal chez les garçons généralement normal chez les garçons ayant présenté une cryptorchidie, mais que la croissance testiculaire pendant la puberté est ralentie – plus particulièrement en cas de cryptorchidie bilatérale et de testicules anciennement non palpables/abdominaux – et s'accompagne d'une diminution de l'inhibine B et d'une légère augmentation de la FSH (avec une testostéronémie globalement similaire), ce qui indique une réduction de la masse des cellules de Sertoli et de la production de cellules germinales. Une orchidopexie précoce (entre 6 et 12 mois) et une descente spontanée sont associées à un volume testiculaire et à des profils endocriniens plus favorables à l'adolescence qu'une correction tardive. Ces observations affinent la stratification des risques et justifient une surveillance pubertaire structurée, basée sur les biomarqueurs, afin d'optimiser le conseil en matière de fertilité.

Mots clés : cryptorchidie, puberté précoce, cellule de Sertoli, traitement

1. Introduction: why puberty matters after cryptorchidism

Cryptorchidism affects ~1–9% of term male newborns globally, with pronounced geographic variation even across closely related Nordic populations [1,5]. In the turn of the millenium, harmonized examinations in Copenhagen (Denmark) and Turku (Finland) demonstrated a roughly four-fold higher prevalence in Denmark than Finland, catalyzing the modern "testicular dysgenesis syndrome" (TDS) discourse spanning impaired fetal Leydig/Sertoli function, cryptorchidism, hypospadias, reduced semen quality, and testicular cancer [1,5–7]. Puberty is the life stage when testicular function recovers (or fails to recover) from earlier

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insults: gonadotropin pulsatility resumes, Sertoli cells complete maturation, the blood-testis barrier consolidates, and spermatogenesis scales up. Consequently, pubertal outcomes in boys with prior cryptorchidism provide an integrated readout of the testis' biological reserve built during fetal life and "mini-puberty" and modified by surgical timing and postnatal environment.

Historically, concerns in cryptorchidism focused on testicular cancer risk and fertility; however, contemporary longitudinal studies show that the **tempo and quality of pubertal testicular growth**—rather than age of pubertal onset—best differentiates formerly cryptorchid boys from controls [2,4]. This review organizes current evidence with an emphasis on Nordic prospective cohorts, laboratory phenotypes, and modifiable determinants of testicular growth trajectories.

2. From fetal life to mini-puberty: setting the stage for adolescence

2.1. Physiology in brief

Fetal testicular differentiation depends on placental hCG acting via the LH/CG receptor before the fetal pituitary–gonadal loop matures; in the second and third trimesters, fetal pituitary LH/FSH takes over steroidogenic regulation. After birth, an HPT "mini-puberty" (weeks 1–16) features rising LH/FSH, peaking total testosterone (~1–3 months), and robust Sertoli-cell outputs (inhibin B, AMH). This surge supports penile growth, scrotal pigmentation, and testis descent completion, but—critically—also expands Sertoli-cell number, which constrains future spermatogenic capacity. Disruption of this window leaves a durable imprint on adolescent fertility potential [2-4, 6-10].

2.2. Mini-puberty in cryptorchid boys: consistent Sertoli-cell signals

Across birth cohorts that sampled at ~3 months (the compromise time point when FSH/inhibin B are near peak, and LH/testosterone are declining from peak), cryptorchid boys—particularly those with higher-lying testes—exhibit **lower inhibin B and/or higher FSH**, consistent with a Sertoli-cell deficit; serum testosterone is often comparable to controls, suggesting relatively preserved Leydig-cell steroidogenesis at a population level [11]. In the joint Danish–Finnish cohorts, mini-puberty hormones differed by testis position: suprascrotal/inguinal and non-palpable testes showed the most adverse Sertoli signals compared with boys whose testes were scrotal at exam [11]. These findings align with the pathophysiology whereby impaired Sertoli proliferation is an early, salient lesion in cryptorchidism, with lasting consequences for pubertal Sertoli mass and inhibin B later on [2–4].

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3. Epidemiology and natural history relevant to puberty

The **Danish–Finnish comparative birth cohorts** quantified marked prevalence differences (e.g., ~9% Denmark vs ~2–3% Finland at standardized exams), provided a robust platform for hormone phenotyping during mini-puberty, and enabled tracking of spontaneous descent [1,2,4-7]. Spontaneous descent commonly occurs by 6 months corrected age; persistence beyond this window predicts need for orchiopexy. Guideline statements (Nordic 2007, AUA 2014, EAU/ESPU 2016) converged on recommending **orchiopexy by 6–12 months** to optimize testicular growth and reduce later risks [3,8,10]. The **timing of orchiopexy** becomes pivotal for pubertal trajectories: early relocation likely preserves more of the Sertoli/germ-cell niche than late surgery, even though puberty itself reactivates gonadotropin support [3,8-10].

4. Puberty after cryptorchidism: what do longitudinal data show?

4.1. Age at pubertal onset and tempo

Multiple cohorts (including Turku and Copenhagen, with standardized anthropometry and repeated testicular volume assessments) indicate that **the calendar age at pubertal onset is not materially delayed** in boys with a history of cryptorchidism compared with peers [2,4]. The more discriminating signal is **testicular growth velocity** once puberty begins. In the Turku study of formerly cryptorchid boys followed across adolescence, testicular volumes at the same Tanner genital stages were **smaller** than in controls, particularly in bilateral and previously non-palpable/abdominal cases [2,4].

4.2. Testicular volume dynamics: unilateral vs bilateral, spontaneous descent vs orchiopexy

Sadov et al. (2016) and Rodprasert et al. (2022) provided a detailed analysis of pubertal testicular growth in formerly cryptorchid boys versus controls, stratified by important clinical modifiers (laterality, whether descent was spontaneous or surgical, and age at orchiopexy). They reported:

- **Onset:** Pubertal onset (clinically defined) was comparable across groups.
- **Growth: Testicular growth during puberty was attenuated** in boys with a history of cryptorchidism; disparities were most pronounced in **bilateral** disease and in those who required surgery (versus spontaneous descent).
- **Endocrine correlates:** Seminiferous growth impairment corresponded to **lower inhibin B** and **higher FSH** during adolescence; LH/testosterone profiles were broadly similar to controls. [2]

These data operationalize the clinical intuition taught in pediatric endocrinology/urology: **puberty does not "fix" a depleted Sertoli pool**; rather, pubertal gonadotropin drive unmasks the anatomic constraint as a blunted volumetric trajectory. Replication and extensions of

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these observations in national registries and clinic-based cohorts support the same direction of effect [10].

4.3. Hormone profiles during adolescence

Sertoli-cell axis (FSH–inhibin B): Relative to controls, formerly cryptorchid adolescents commonly show **higher FSH** and **lower inhibin B**, pointing to suboptimal Sertoli mass/germ-cell output. Differences scale with bilateral disease, non-palpable position, and later surgery [2]. This **discordant Sertoli signal in the face of normal or near-normal testosterone** is the hallmark endocrine phenotype and has direct fertility implications.

Leydig-cell axis (LH–testosterone): Most cohorts report **no major difference in serum testosterone** at comparable pubertal stages; LH can be slightly higher on average in bilateral/operated groups, but within reference ranges [2]. This suggests that Leydig-cell function—at least as captured by circulating testosterone under pubertal LH drive—is relatively preserved in many formerly cryptorchid boys, even when seminiferous expansion lags.

AMH: Pubertal AMH normally falls as Sertoli cells mature under intratesticular testosterone. AMH patterns in formerly cryptorchid cohorts are heterogeneous, reflecting the interplay of delayed Sertoli maturation and reduced Sertoli mass; AMH is generally **not** the preferred monitoring biomarker in mid-to-late puberty compared with inhibin B/FSH. [2,3,12]

4.4. Growth references and measurement methods

Testicular volume is best tracked **longitudinally** using the same method. Orchidometry (Prader beads) remains widely used, but **ultrasound** provides more precise estimates and can detect modest inter-testis asymmetries important in unilateral disease. Scandinavian pubertal studies have often combined both methods to strengthen inferences and added also ruler measurements [2,4,13].

5. Determinants of pubertal outcome after cryptorchidism

5.1. Laterality and initial position

Bilateral cryptorchidism predicts **smaller pubertal testicular volumes**, **lower inhibin B**, and **higher FSH** compared with unilateral cases and controls [2,4,13]. Within each laterality stratum, **higher-lying/non-palpable** testes have worse trajectories than suprascrotal/low-inguinal positions, consistent with the gradient of thermal and developmental insult inferred in infancy [2,4,13,14].

5.2. Spontaneous descent vs orchiopexy

Boys with **spontaneous descent** generally perform better in adolescence than those who required **orchiopexy**, supporting the concept that spontaneous descent is a **biomarker of a more competent testis** at baseline. Among the surgically corrected, earlier operation is associated with **better pubertal testicular growth and Sertoli-axis labs** [13,14].

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5.3. Timing of orchiopexy

Nordic and European guidelines recommend **orchiopexy between 6 and 12 months**, certainly before 18 months, to optimize later testicular growth and fertility potential [3,10]. Adolescent follow-ups confirm that **earlier repair** tracks with **larger pubertal testicular volumes** and **more favorable inhibin B/FSH** than late repair, though surgery cannot fully normalize outcomes in bilateral/non-palpable disease [3,8,13,14].

5.4. Mini-puberty phenotype as a predictor

Inhibin B and FSH measured at ~3 months stratify later risk: **lower inhibin B/higher FSH** in cryptorchid infants (especially in those with higher-lying testes) predict more attenuated pubertal seminiferous growth and a higher likelihood of adverse adolescent Sertoli-axis profiles [2,13]. Such associations bind infancy to adolescence and rationalize early endocrine testing in persistent cryptorchidism.

6. Mechanistic links: from dysgenesis to pubertal shortfall

Two nonexclusive mechanisms bridge cryptorchid infancy to pubertal outcomes:

1. **Primary testicular dysgenesis** (impaired Sertoli/germ-cell programing), reflecting fetal/placental endocrine disruption and/or gene–environment risk. This manifests as **deficient mini-puberty Sertoli signals** (inhibin B), constraining the maximal proliferative base of the seminiferous epithelium available at puberty [2,13].
2. **Secondary heat/ischemic injury** from extra-scrotal position, proportional to height (inguinal → abdominal), duration, and laterality, which cumulatively **erodes germ-cell number** and Sertoli mass. Earlier relocation limits dose–time exposure and better preserves the prepubertal platform for pubertal expansion [3,8,14].

At puberty, GnRH/LH/FSH activation drives Leydig testosterone and FSH-mediated Sertoli support. If the Sertoli-cell pool is small, **FSH rises** and **inhibin B remains low** despite normal LH/testosterone, and testicular volume grows along a shallower slope—**the quintessential cryptorchid adolescent signature** [2,3,13].

7. Clinical implications for pubertal care

7.1. Who needs structured follow-up?

All boys with **bilateral** cryptorchidism, **non-palpable/abdominal** testes, **late orchiopexy** (>12–18 months), or **abnormal mini-puberty labs** warrant **structured adolescent follow-up** (growth, genital staging, testicular volume, and selective labs). Unilateral low-inguinal cases repaired early and boys with spontaneous descent have lower risk but still merit counselling and a **baseline pubertal assessment**. [2,14]

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7.2. What to measure and when?

- **Testicular volume** (orchidometer ± ultrasound) during puberty, noting symmetry in unilateral cases.
- **FSH and inhibin B** at mid-puberty (e.g., Tanner G3–G4) to appraise Sertoli axis; consider repeat in late puberty if values are borderline.
- **LH/testosterone** only if clinical signs suggest hypoandrogenism; routine measurement is less discriminative.
- **AMH** is mainly informative prepubertally and in the early stages of puberty; interpret with caution later on. [12]

7.3. Counselling about fertility

The adolescent phenotype (smaller testicular volumes, low inhibin B/high FSH) correlates with reduced adult semen quality in population studies; bilateral cryptorchidism and late surgery carry the **highest odds of oligo/azoospermia**. Early orchiopexy and spontaneous descent are favorable signs but **do not guarantee normal fertility**, especially after bilateral disease. Counselling should be realistic and individualized, and in late adolescence/young adulthood, **semen analysis** provides the definitive assessment. [3,8,10]

8. Controversies and knowledge gaps

- **Biomarker thresholds:** While group differences are consistent, **individual-level** cut-offs for inhibin B/FSH that predict future infertility lack universal validation.
- **Leydig-cell reserve:** Subtle androgen deficiencies may not be captured by total testosterone alone; studies using **free testosterone** or **androgen bioactivity** assays are limited and need replication.
- **Ethnic/environmental heterogeneity:** The Danish–Finnish contrast underscores environmental contributions, but the **specific exposures** remain incompletely resolved.
- **Surgery alone vs adjuvant therapy:** Whether peri-operative hormonal therapy improves adolescent Sertoli outcomes remains debated; current practice prioritizes **timely orchiopexy** over GnRH/HCG regimens, given mixed evidence and concerns over down-stream fertility endpoints. [3,8,10]

9. Practical algorithm for adolescent follow-up (risk-tiered)

1. **Risk stratification at or after repair:**
    - *Low*: unilateral, low-inguinal, spontaneous descent or orchiopexy ≤12 months, normal mini-puberty labs.

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- *Intermediate*: unilateral high-inguinal or non-palpable, orchiopexy 12–18 months, unavailable infant labs.
- *High*: bilateral, non-palpable/abdominal, orchiopexy >18 months or staged, abnormal infant inhibin B/FSH.

2. **Monitoring plan:**
    - *Low*: clinical pubertal review; testicular volume at G2/G3; labs if volumes small for stage.
    - *Intermediate*: testicular volumes + **FSH/inhibin B** at G3; repeat at G4–G5 if abnormal.
    - *High*: repeated testicular volumes through mid-puberty; **FSH/inhibin B** at G2–G3 and G4; consider ultrasound at baseline.
3. **Transition planning:**
    - Late-adolescent/young adulthood **semen analysis** for intermediate/high-risk groups; endocrine referral if **FSH persistently high/inhibin B low** or volumes plateau <15–20 mL combined.

This pragmatic approach operationalizes what the longitudinal literature—especially the Copenhagen/Turku experiences—has taught us about the **shape** of pubertal development after cryptorchidism. [1-4,6,13,14]

10. Conclusion

Boys with a history of cryptorchidism, especially **bilateral** or **non-palpable** disease and those repaired **after** the first year of life, typically enter puberty at the **usual age** but show **constrained testicular growth** and a **Sertoli-axis signature** (low inhibin B, elevated FSH) consistent with reduced seminiferous capacity. These pubertal findings echo endocrine phenotypes already visible during **mini-puberty**, binding fetal–neonatal events to adolescent outcomes. Early orchiopexy and spontaneous descent are favorable modifiers but do not fully normalize pubertal trajectories in higher-risk subgroups. A risk-tiered, biomarker-informed adolescent follow-up can sharpen counselling about fertility and guide timely referral for semen analysis and adult-care transition. Continued collaboration across Nordic and international cohorts will refine individualized prediction—transforming the epidemiologic signal of cryptorchid infancy into actionable pubertal care.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

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c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding: see manuscript 4,10,12

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Editorial comment

Impaired mini puberty with decreased LH and testosterone levels affected Ad and Sertoli cell development through positive and negative regulation of morpho regulatory and apoptotic genes. GnRHa treatment had a repressive effect on most Sertoli cell specific genes, which suggested that Sertoli cells underwent a cellular rearrangement. Gonadotropin-dependent increases in FASLG and GDNF expression drove Sertoli cell proliferation and germ cell self-renewal and supported the transition of gonocytes to Ad spermatogonia, independent of inhibin. Furthermore, reductions in RNA expression of *CDKN1B, CALB2, CTSL, CREB1, DMRT1,* and *WT1* occurred. All these genes are involved in Sertoli cell function and differentiation. This finding suggested that GnRHa induced transcriptional changes in multiple target genes. (Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, Dasevicius D, Stadler MB et al.) Curative GnRHa treatment has an unexpected repressive effect on Sertoli cell specific genes. Basic Clin Androl. 2018;28:2.1-10.)

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Roles of temperature and retinoic acid in the spermatogenesis defect associated with cryptorchidism

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Abstract

Spermatogenesis is an evolutionarily conserved but physiologically fragile process that requires the testis to operate within a tightly controlled environment. Most mammals externalize their testes into the scrotum, maintaining a cooler temperature than the abdominal cavity. Failure of testicular descent—cryptorchidism—exposes the testis to higher temperatures and may cause defects in spermatogenesis and male infertility. Yet, it has remained elusive how high temperature affects spermatogenesis, and whether heat is the sole causal factor of spermatogenic defects in cryptorchidism. A recent study from our group, using an ex vivo organ culture system, revealed that temperature elevations of only 1–2 °C induce stage-specific blocks in spermatogenesis. In particular, meiotic double-strand break repair was found to be temperature-sensitive, triggering checkpoint-mediated germ cell elimination. We also showed that heat alone does not fully explain the pathology of cryptorchid testis: While all germ cells but undifferentiated spermatogonia are depleted in an artificial cryptorchid testis model—in which testes are exposed to 38 °C—such histological features were not observed in seminiferous tubules cultured at 38 °C or any other temperature tested. Further, we found that intratesticular retinoic acid (RA) levels decline within 40–48 h following testis translocation without overt cell loss, and that exogenous RA could restore spermatogonial differentiation even under body core temperature. Together, these findings indicate that cryptorchid-associated spermatogenesis defects may result from a combined insult, with high temperature impairing meiosis and spermiogenesis, and with RA depletion compromising spermatogonial commitment. Understanding this dual mechanism will open new translational opportunities, including potential therapies targeting RA metabolism.

Key words Spermatogenesis, temperature adult testis, experimental cryptorchidism

Résumé

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La spermatogenèse est un processus évolutivement conservé mais physiologiquement fragile, nécessitant un environnement testiculaire strictement contrôlé. Chez la plupart des mammifères, les testicules sont externalisés dans le scrotum afin de maintenir une température plus basse que celle de la cavité abdominale. L'échec de la descente testiculaire—cryptorchidie—expose le testicule à une chaleur excessive et peut entraîner des altérations de la spermatogenèse et une infertilité masculine. Les mécanismes précis par lesquels la chaleur perturbe la spermatogenèse, et la question de savoir si la température élevée est l'unique facteur causal dans la cryptorchidie, demeuraient toutefois incertains.

Une étude récente de notre groupe, utilisant la culture organotypique ex vivo, montre que des élévations de seulement 1–2 °C induisent des blocages stade-spécifiques de la spermatogenèse. La réparation des cassures double-brin méïotiques apparaît particulièrement thermo-sensible, entraînant une élimination checkpoint-dépendante des cellules germinales. Nous démontrons également que la chaleur ne suffit pas à reproduire l'histopathologie de la cryptorchidie : alors que tous les types cellulaires germinatifs sauf les spermatogonies indifférenciées sont déplétés dans un modèle expérimental de cryptorchidie (38 °C), ce profil n'apparaît pas dans les tubes séminifères cultivés à 38 °C ou à toute autre température testée. De plus, les niveaux d'acide rétinoïque (AR) intratesticulaire chutent 40–48 h après la translocation testiculaire, avant toute perte cellulaire apparente, et l'apport exogène d'AR restaure la différenciation spermatogoniale même à température corporelle.

Ces résultats indiquent que les défauts de spermatogenèse associés à la cryptorchidie résultent d'une agression combinée : la chaleur perturbe la méiose et la spermiogenèse, tandis que la déplétion en AR compromet l'engagement spermatogonial. La compréhension de ce double mécanisme ouvre des perspectives translationnelles, notamment des thérapies ciblant le métabolisme de l'acide rétinoïque.

Mots-clés: Spermatogenèse, température du testicule adulte, cryptorchidie expérimentale

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Introduction

Most mammals position the testes in the scrotum, where temperature is maintained below core body temperature [1,2]. This anatomical adaptation is widely thought to protect spermatogenesis by sustaining a cooler milieu. By contrast, some mammals (e.g., cetaceans and proboscideans) lack a scrotum but have evolved alternative testicular cooling strategies [2]. These comparative observations underscore temperature control as a central principle of male germ-cell development.

Our laboratory aims to understand spermatogenesis with a particular emphasis on stem cell regulation in the adult testis. [3-5]. A persistent question is how temperature modulates the balance between spermatogonial stem cell (SSC) self-renewal and differentiation, and how subsequent stages—meiosis and spermiogenesis—respond to small thermal shifts. Cryptorchidism provides a clinically relevant lens for this question but is confounded in vivo by extratesticular factors (endocrine and neural inputs) and the difficulty of precisely measuring or controlling intratesticular temperature [6]. We therefore combined an **artificial cryptorchidism model** with an **ex vivo organ culture system** to dissect temperature-dependent steps and to assess whether heat alone accounts for the cryptorchid phenotype.

A guiding observation from artificial cryptorchidism is that after translocating adult mouse testes from the scrotum (~34 °C) to the abdominal cavity (~38 °C), seminiferous tubules become depleted of differentiating germ cells, retaining primarily **undifferentiated spermatogonia** (Aundiff) alongside Sertoli cells. **KIT–positive** differentiating spermatogonia are absent in this condition [6]. This suggested—initially—that high temperature may block the **commitment** of Aundiff to the differentiating spermatogonia. The work summarized here tests that hypothesis, identifies **stage-specific thermal sensitivities**, and reveals an additional, temperature-linked **retinoic acid (RA)** deficiency that helps explain the full cryptorchid pathology [7-10].

Methods and experimental systems

Artificial cryptorchidism in adult mice

We performed surgical translocation of testes from the scrotum to the abdominal cavity (adjacent to the liver), after normal adult spermatogenesis had been established. Intratesticular temperature increased from ~34 °C to ~38 °C (directly measured). Within this "extreme" but controlled model, histology consistently showed shrunken seminiferous tubules largely devoid of germ cells beyond Aundiff, consistent with previous reports and mirroring classic reports of cryptorchid pathology [6].

Ex vivo organ culture of seminiferous tubules

To isolate temperature as a sole controlled parameter and to exclude systemic influences, we employed a long-term **gas–liquid interface organ culture** of seminiferous tubules (based on an established by Takehiko Ogawa, Yokohama Japan) [11,12]. Cultures at **34 °C** supported full spermatogenesis, including undifferentiated and differentiating spermatogonia, pachytene spermatocytes, round spermatids, and elongated spermatids, although being compromised compared with physiological spermatogenesis occurring in vivo. We then adjusted incubator

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setpoints in 1–2 °C increments to test a narrow temperature window from ~32 °C to 38 °C, scoring outcomes by histology, immunohistochemistry (including KIT and a meiosis marker Sycp3, late meiotic and haploid cell marker Protamine-GFP transgene).

Readouts for meiotic integrity and RA signaling

We evaluated meiotic progression (e.g., completion of meiosis I, appearance of haploid cells) and assessed **DNA double-strand breaks (DSBs),** through chromosome spread methods combined with immunostaining for DSBs (γH2AX) and meiosis machinery proteins (e.g., SCP1, SCP3, RPA2, RAD51, DMC1, and MLH1) and apoptosis proteins (cleaved Caspase 3) [13]. To assess the role of RA signaling, we measured intratesticular RA levels after testis translocation, profiled expression of key metabolic enzymes by qRT-PCR, and tested whether exogenous retinol/RA supplementation **c**ould rescue differentiation in the cryptorchid milieu.

Results

Temperature produces stage-specific **blocks rather than a single threshold effect** [13].

Contrary to our initial expectation of a single "commitment block of spermatogonial stem cells," we observed a **stepwise, stage-specific pattern** of thermal sensitivity:

- **32–35 °C:** Spermatogenesis proceeded well to the stage of **elongated spermatids**.
- **~36 °C:** Elongated spermatids were lost, but **round spermatids** persist, indicating a selective vulnerability of **spermiogenesis** before complete arrest of meiosis.
- **~37 °C:** Failure to complete **meiosis** is evident; haploid cells were absent.
- **~38 °C:** Spermatocytes did not progress beyond **mid-pachytene** stages; meiotic progression was effectively blocked.

These observations demonstrate that increases of only **1–2 °C** can shift the dominant defects from **spermiogenesis** (block of spermatid elongation) to **meiotic arrest**, leading to non-uniform thermal sensitivities across germ-cell stages.

High temperature impairs meiotic DSB repair and induces apoptosis on meiotic checkpoint

At 37–38 °C in culture, meiotic cells showed evidence of **increased unrepaired DSBs** persisting into pachytene stage, likely triggering **meiotic checkpoint–mediated elimination** of damaged germ cells. This mechanistic link—DSB repair sensitivity to heat and checkpoint activation—accounts for the abrupt **pachytene-stage arrest** and loss of downstream haploid cells at higher temperatures. [6,13].

Heat alone does NOT cause the full artificial cryptorchid pathology [6,13].

Despite recapitulating substantial defects in meiosis and spermiogenesis at 37–38 °C ex vivo, we found that the organ culture **never** reproduced the **complete depletion of differentiating germ cells** as seen in artificial cryptorchidism (i.e., "Sertoli cell–only" with residual undifferentiated spermatogonia and absence of KIT–positive differentiating spermatogonia

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and more advanced cells). Thus, while heat clearly disrupts meiosis and spermiogenesis, it **does not fully explain** the cryptorchid testis histology.

Cryptorchid testes rapidly lose intratesticular RA before cell loss

In the artificial cryptorchid model, **RA levels decline** within 2 days after translocation to the abdomen, before **ov**ert cell loss observed in 3–5 days. This **early RA depletion** occurs while all major cell types—including **pachytene spermatocytes**, the predominant RA producers via aldehyde dehydrogenase enzymes—are still present. Bulk RNA-seq before/after translocation did **not** reveal a clear, systematic change in RA-metabolizing enzyme **mRNA** levels, suggesting that the drop in RA could arise from **post-transcriptional** mechanisms.

Exogenous RA triggers spermatogonial commitment at body-core temperature

Administration of retinol, an RA precursor, to mice carrying cryptorchid testes could trigger differentiation of undifferentiated spermatogonia into differentiating spermatogonia. This occurred **under body-core temperature (~38 °C)**, indicating that **high temperature does not prevent spermatogonial commitment** per se if adequate RA signaling is available.

Discussion

Multiple causes for spermatogenic defects in artificial cryptorchid

Our data indicate the pathophysiology of artificial cryptorchid testis involves multiple causes:

1. **Thermal injury to meiosis and spermiogenesis:**
   A rise from 34 °C to 36–38 °C is sufficient to sequentially compromise (i) spermiogenesis (loss of elongation at ~36 °C) and (ii) meiotic completion (arrest and elimination at ~37-38 °C). In particular, **DSB repair** is **impaired** in meiotic prophase I, leading to **apoptosis-mediated germ-cell elimination** [13].

2. **Lowered RA compromising spermatogonial commitment:**
   Artificial cryptorchidism triggers a **rapid decrease in intratesticular RA**, compromising undifferentiated-to-differentiating spermatogonial transition before histological degeneration, resulting in tubules with Sertoli cells and undifferentiated spermatogonia but no other differentiating germ cells. **Exogenous RA** can overcome this block even at 38 °C, showing that **RA shortage—not heat alone—contributes to the spermatogenesis defect.**

Implications for basic biology

The **fine granularity** of thermal sensitivity (1–2 °C steps) argues that spermatogenesis is buffered only within a **narrow thermal optimum,** highlighting **distinct thermal liabilities** of cellular programs (chromosomal synapsis formation, recombination, checkpoint control). The observation that RA levels fall quickly **without** corresponding mRNA changes for RA metabolic enzymes points to **non-transcriptional control** of RA homeostasis potentially involves enzyme kinetics and other mechanisms.

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Implications for human pathology and therapeutic strategies

This study may provide some practical implications for human cryptorchidism. In particular, targeted modulation of RA metabolism or RA delivery may help preserve or restore some spermatogenesis. However, from a practical perspective, patient will receive orchiopexy before spermatogenesis proceeds upon puberty. In addition, the artificial cryptorchid mouse model is an extreme and controlled setting, making translation to human pathology require caution.

Conclusions

Spermatogenesis exhibits narrow thermal tolerance with distinct, stage-specific sensitivities: spermiogenesis is compromised near 36 °C, meiosis fails by ~37-38 °C through heat-sensitive DSB repair and meiotic checkpoints. Heat alone does not account for the full cryptorchid pathology. Instead, rapid decrease in RA concentration following testis translocation prevents spermatogonial commitment, producing the characteristic depletion of differentiating germ cells. Exogenous RA rescues this commitment step at body-core temperature, pointing to RA metabolism as a potential translational target. We propose a dual-insult mechanism in cryptorchidism: temperature deranges meiosis/spermiogenesis, while RA deficiency arrests spermatogonial differentiation.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Altered DNA methylation in estrogen-responsive repetitive sequences of spermatozoa of infertile men with shortened anogenital distance

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Abstract

Background: Anogenital distance (AGD) is a sexually dimorphic, life-long anthropometric marker that reflects androgen/estrogen balance during a critical fetal "masculinization programming window." Shorter male AGD has been associated with cryptorchidism, hypospadias, impaired semen quality and reduced testicular volume—core features of the testicular dysgenesis syndrome (TDS) hypothesis. Circumstantial evidence suggests that prenatal exposure to endocrine disruptors is involved in causing TDS. Many prevalent endocrine-disrupting chemicals (EDCs) act through the estrogen receptor (ER) and can perturb fetal programming. How a transient prenatal exposure could leave durable molecular traces into adult spermatogenesis remains a key mechanistic question. A plausible mechanism is epigenetic reprogramming: in the postimplantation embryo human primordial germ cells (PGCs) undergo genome-wide DNA demethylation and subsequent re-methylation; during this window, transposable elements—particularly primate-specific Alu repeats—can be de-/re-methylated and many harbor ER-responsive sequence motifs.

Objectives: Building on clinical work from Basel we synthesize evidence and present a working model in which infertile men with shortened AGD harbor distinctive DNA-methylation patterns at estrogen-responsive Alu elements in sperm, especially within subpopulations marked by chromatin immaturity (CMA3 positivity) or apoptosis (YO-PRO-1 positivity). We outline study design, summarize core findings, and discuss translational implications and limitations.

Methods (conceptual framework): Men undergoing fertility evaluation were phenotyped (including AGD and testicular volume). Ejaculates with adequate counts were processed by swim-up and sorted by flow cytometry into fractions with high vs. low chromatin decondensation (chromomycin A3, CMA3) and with vs. without early apoptotic membrane permeability (YO-PRO-1). Reduced-representation bisulfite sequencing (RRBS) profiled CpG methylation genome-wide; bioinformatics focused on repetitive elements (Alu) containing canonical/pseudo-ERE motifs and ER-ChIP–supported sites.

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Results (integrative summary): Across unsorted sperm, global CpG methylation distributions were broadly similar between infertile men (short AGD) and fertile donors. In sorted fractions, however, infertile men exhibited (i) enrichment of **hypomethylated** Alu-EREs within CMA3-positive and YO-PRO-1–positive sperm, and (ii) a deficit of **hypermethylated** Alu-EREs relative to fertile controls. These shifts were most evident in the "abnormal" subpopulations, consistent with histone retention/protamine deficiency and apoptotic signaling.

Conclusions: We propose that prenatal estrogenic/antiandrogenic perturbation (indexed in adulthood by shortened AGD) is traceable in sperm as altered methylation of ER-responsive repetitive elements. Such lesions concentrate in spermatozoa with defective chromatin maturation or incipient apoptosis, potentially contributing to reduced fertilizing competence and intergenerational epigenetic risk. We discuss how this model aligns with TDS and outline paths for validation (orthogonal epigenomics, si,ngle-cell assays, environmental exposure reconstruction).

Key words Cryptorchidism, ano-genital distance, estrogen

Résumé

Contexte. La distance anogénitale (AGD) est un marqueur anthropométrique sexuellement dimorphique reflétant l'équilibre androgènes/estrogènes durant la fenêtre critique de masculinisation fœtale. Une AGD masculine réduite est associée à la cryptorchidie, l'hypospadias, une qualité spermatique diminuée et un faible volume testiculaire, éléments centraux du syndrome de dysgénésie testiculaire (TDS). Plusieurs perturbateurs endocriniens (EDC) prévalents agissent via le récepteur des estrogènes (ER) et peuvent affecter la programmation fœtale. Un mécanisme plausible reliant une exposition prénatale transitoire à des altérations durables de la spermatogenèse est la reprogrammation épigénétique des cellules germinales primordiales, notamment au niveau des éléments transposables Alu, riches en motifs réactifs à l'ER.

Objectifs. Sur la base de travaux cliniques menés à Bâle, nous proposons un modèle où les hommes infertiles présentant une AGD courte montrent des profils distinctifs de méthylation de l'ADN au niveau d'éléments Alu sensibles aux estrogènes dans leurs spermatozoïdes, en particulier dans les sous-populations immatures (CMA3 positives) ou apoptotiques précoces (YO-PRO-1 positives).

Méthodes. Des hommes évalués pour infertilité ont été phénotypés (AGD, volume testiculaire). Les échantillons spermatiques ont été enrichis (swim-up) puis triés par cytométrie selon la condensation chromatinienne (CMA3) et la perméabilité membranaire apoptotique (YO-PRO-1). Le RRBS a profilé la méthylation CpG, avec une analyse ciblée des éléments Alu contenant des motifs ERE.

Résultats. Dans les spermatozoïdes non triés, les profils globaux de méthylation étaient comparables entre hommes infertiles (AGD courte) et donneurs fertiles. En revanche, dans les fractions triées, les hommes infertiles présentaient un enrichissement en Alu-ERE

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hypométhylés et un déficit en Alu-ERE hyperméthylés dans les sous-populations CMA3 positives et YO-PRO-1 positives.

Conclusions. Une perturbation prénatale estrogénique/anti-androgénique, reflétée à l'âge adulte par une AGD courte, pourrait laisser une empreinte durable dans la méthylation d'éléments répétitifs sensibles aux estrogènes dans le sperme. Ces anomalies, concentrées dans les spermatozoïdes à maturation chromatinienne défectueuse ou en apoptose débutante, pourraient contribuer à une baisse de compétence fécondante et à un risque épigénétique intergénérationnel. Des pistes de validation sont proposées.

Mots-clés: Cryptorchidie, distance ano-génitale, œstrogène

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Introduction

The **testicular dysgenesis syndrome (TDS)** hypothesis posits that a spectrum of male reproductive disorders—cryptorchidism, hypospadias, low sperm counts/poor semen quality and testicular germ cell cancer—share origins in disturbed fetal testis development driven by gene–environment interactions [1,2]. A practical anthropometric proxy of that early hormonal milieu is **anogenital distance (AGD)**, which is longer in males, established in utero, and trackable across life [3–6]. In humans, shorter male AGD has been associated with cryptorchidism and hypospadias in infancy, and with lower semen quality and reduced testicular volume in adulthood [3–6]. (For contemporary overviews linking AGD with male reproductive outcomes see [3–6].)

Many **endocrine-disrupting chemicals (EDCs)** with widespread human exposure (e.g., phthalates, bisphenol A) can act via estrogen receptor pathways or antiandrogenic mechanisms during the fetal masculinization window, and several birth cohort studies associate prenatal phthalate exposure with shorter male AGD [7–12].

A critical mechanistic puzzle is: **How do transient fetal exposures produce enduring molecular changes** that persist into adult spermatogenesis? One compelling answer is **epigenetic reprogramming** in the postimplantation embryo. During weeks 7–17 of human development, PGCs undergo profound, genome-wide DNA demethylation followed by sex-specific re-methylation; transposable elements show distinctive and dynamic methylation behavior during this time [13–15].

Notably, **transposable elements (TEs)**—especially primate-specific **Alu** repeats—are not inert "junk," but major contributors to the mammalian regulatory landscape; numerous transcription factor binding sites, including those for **estrogen receptor α (ESR1)**, derive from TEs [16–18] If prenatal estrogenic signaling targets TE-borne ER response elements (EREs) during the PGC reprogramming window, **persistent, locus-selective methylation scars** could plausibly be carried forward into adult sperm.

Here we integrate a Basel clinical study framework with the broader literature to examine whether **infertile men with shortened AGD** harbor **altered DNA methylation at ER-responsive repetitive elements** in sperm, and how such alterations relate to **chromatin maturity** and **apoptosis** at the single-cell fraction level [19].

Background and Rationale

AGD as a biomarker of fetal androgen/estrogen balance

AGD is established by the **androgen surge** in the masculinization programming window and remains sexually dimorphic throughout life. Epidemiologic studies in men link **shorter AGD** to **poorer semen quality** and **lower testicular volume**, while pediatric studies associate shorter AGD with **cryptorchidism** and **hypospadias** [3–6].

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Endocrine disruptors that impinge on ER signaling and AGD

Prenatal phthalate exposure has been associated with shorter AGD in boys (notably in U.S. cohorts), and BPA/other EDCs can **signal through ERα/ERβ** with genomic actions at EREs and non-genomic membrane-initiated cascades [7–12, 20–22]. This fits TDS, where fetal testis endocrine dysfunction seeds later dysfunctions [1,2].

Why repetitive DNA?

Repetitive elements (e.g., **Alu**, LINE-1) are abundant in the human genome. TEs **seed transcription-factor binding motifs** and shape regulatory innovation [16–18]. Crucially, many ER binding sites in human cells map to TE-derived sequences; **ERE-like motifs** are **over-represented in Alu** families, providing a substrate for estrogen-dependent regulation and potential targets for EDCs [16–18].

The PGC reprogramming window

Human PGCs undergo near-global **DNA methylation erasure** followed by sex-specific re-methylation during fetal development, with **TEs displaying family-specific dynamics** [13–15]. If estrogenic/antiandrogenic signals alter the establishment of methylation at **Alu-ERE loci**, such changes could **persist into the adult germline**, making mature sperm a biospecimen to read out those early events.

Clinical Study Overview (Basel): Phenotyping, Sorting and Methylome Profiling

Participants and clinical phenotyping

Infertile men were recruited from a university fertility clinic; **AGD** (anus-to-scrotum) and **testicular volume** were measured. Fertile sperm donors served as controls. Because downstream assays required sufficient cell numbers, men with **normozoospermia** or mild oligozoospermia were preferentially included. **Shortened AGD** defined the infertile patient group, consistent with TDS epidemiology [3–6].

Sperm sub-fractionation by functional staining

To reduce heterogeneity, ejaculates were **sorted by flow cytometry** into subpopulations using:

- **Chromomycin A3 (CMA3)**—a fluorochrome that competes with protamines for DNA binding. High CMA3 staining indicates **protamine deficiency / histone retention** and is linked to poor chromatin condensation [23].
- **YO-PRO-1**—an early-apoptosis marker permeating cells with compromised membranes; in human sperm it correlates with **DNA fragmentation** and apoptotic features [24].

DNA methylation assay

Genome-wide CpG methylation was profiled by **reduced-representation bisulfite sequencing (RRBS)**—an established, cost-effective method enriching for CpG-dense regions

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while preserving quantitative methylation calls at single-CpG resolution [25–27]. Bioinformatics focused on **Alu repeats** carrying **canonical/pseudo-ERE motifs** and, when available, ER ChIP-seq-supported sites (to enrich for bona fide estrogen-responsive repeats). The **primary contrasts** were between **infertile men (short AGD)** versus **fertile donors**, both **globally** and **within sorted subfractions** (CMA3^high/low and YO-PRO-1^pos/neg).

Key Findings (Integrated Summary)

1. **Global methylation:** When all sperm were analyzed without regard to subfraction, **global CpG methylation distributions** were broadly similar between infertile and fertile men—unsurprising given the dilution of subtle, cell-state–specific signals by population averaging.
2. **ERE-bearing Alu elements:** Within **CMA3-positive** (chromatin-immature) fractions from **infertile men with short AGD**, **hypomethylated Alu-EREs** were **significantly enriched**, whereas **hypermethylated Alu-EREs** were **under-represented** relative to fertile controls. Analogous patterns were seen in **YO-PRO-1–positive** (apoptosis-prone) fractions.
3. **Functional link:** The **co-localization** of altered methylation at **ER-responsive repetitive loci** with **chromatin immaturity** and **apoptotic signatures** suggests that **epigenetic scars of ER signaling** (potentially laid down in fetal life) are **preferentially carried** by sperm subpopulations with **reduced fertilizing potential**.

These observations provide a mechanistic bridge among **short AGD**, **EDC/ER signaling**, and **sperm functional deficits**, consistent with TDS. (For background linking AGD to adult semen/testis phenotypes see [3–6]; for TE-derived ER regulatory sites see [16–18]; for CMA3/apoptosis markers see [23,24].

Mechanistic Interpretation

A prenatal "hit" recorded in repetitive DNA

During PGC reprogramming, **TEs undergo dynamic methylation**. If the embryo/fetus experiences **estrogenic/antiandrogenic perturbation** (e.g., phthalates, BPA), **ER signaling** may transiently bind **ERE-like motifs within Alu repeats**, locally affecting **de novo methyltransferase access/activity**. The result would be **stable hypomethylation/hypermethylation** at specific **Alu-ERE loci** that persist into spermatogenesis. In adulthood—especially under suboptimal testicular environments consonant with the TDS spectrum—those loci may **co-segregate** with **spermatozoa showing chromatin remodeling defects (CMA3^high)** or **apoptotic features (YO-PRO-1^pos)**, amplifying functional consequences.

Why the signal concentrates in CMA3+ and YO-PRO-1+ subfractions

Spermiogenesis involves **histone–protamine exchange**; failures in this process yield **protamine deficiency** and **retained histones**, captured by **CMA3** staining and linked to

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impaired DNA packaging and fertility [23]. Apoptotic signaling in sperm (YO-PRO-1 positivity) correlates with **DNA fragmentation** and reduced fertilizing capacity [24]. If **Alu-ERE methylation lesions** predispose to **faulty chromatin remodeling** or heightened DNA damage response during spermiogenesis, they would **enrich** within these "abnormal" subpopulations.

TDS context

Short AGD has been associated with cryptorchidism/hypospadias and impaired semen quality/testicular size, consistent with **TDS** [1–6]. The **Alu-ERE methylation signature** offers a **molecular substrate** for that syndrome—an imprintable, **ER-tunable repetitive element network** perturbed during the **fetal masculinization window** and expressed later as **sperm functional deficits**.

Methodological Considerations

Phenotyping

Standardized **AGD** measurement protocols, blind to case/control status, minimize misclassification. Notably, **AGD is only weakly related to adult BMI** and can be reliably measured across adulthood [4–6]. **Testicular volume** by ultrasound/orchidometer provides a concurrent functional correlate.

Cell sorting and markers

CMA3 and YO-PRO-1 are **widely used markers** in andrology laboratories: **CMA3** positively correlates with **protamine deficiency** and adverse semen parameters [23]; **YO-PRO-1** is validated as a **sperm apoptosis/early membrane compromise** marker with associations to DNA fragmentation [24].

RRBS and repetitive methylomes

RRBS captures CpG-rich portions of the genome and, when paired with repeat-aware mapping, can robustly quantify methylation within **Alu** subfamilies [25–27]. Orthogonal methods (e.g., **targeted bisulfite amplicon sequencing** at sentinel Alu-EREs; **Nanopore methyl-calling** spanning repeats) can validate index findings.

Bioinformatics focus on ERE-bearing Alu

Alu consensus contains **ERE-like motifs**; multiple ER cistromes show strong enrichment in TEs [16–18]. Filtering Alu instances by **ERE motif quality** and **ER ChIP support** increases specificity for estrogen-responsive elements while controlling for the high copy number of Alu.

Clinical and Translational Implications

1. **Etiologic insight:** A **short AGD** in an infertile man is not just an anatomic curiosity; it plausibly **indexes fetal endocrine disruption** and, as shown here, may co-occur with

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molecular scars in sperm at **ER-responsive repeats**. This accords with epidemiologic links between AGD and semen quality/testicular volume [3–6].

2. **Biomarker development:** A **composite biomarker** combining **AGD**, **sperm subfraction proportions** (CMA3^high, YO-PRO-1^pos), and **Alu-ERE methylation indices** could stratify **idiopathic male infertility** and **ART prognosis**.
3. **Exposure reconstruction and prevention:** Although direct prenatal exposure data are rarely available decades later, **molecular readouts** (e.g., **ERE-Alu methylation**) might help **reconstruct exposure histories** at a class level (estrogenic vs. antiandrogenic). This could motivate public-health measures aimed at **reducing EDC exposure** during pregnancy [7–12,20–22].
4. **Intergenerational considerations:** TE-proximal methylation changes in sperm raise questions about **embryo/placental gene regulation** at implantation and **offspring health**—areas suited to **prospective preconception cohorts** and **embryo-derived tissue** studies.

Limitations

- **Sample size and age:** Clinical constraints limit recruitment; infertile adults are often older than fertile donors, a potential confounder—though methylation contrasts were **subfraction-specific** rather than global.
- **Exposure misclassification: Short AGD** is a **proxy**, not proof, of prenatal EDC exposure; contemporaneous fetal biospecimens are unavailable.
- **Marker specificity: CMA3** is a proxy for protamine deficiency; **YO-PRO-1** marks early apoptosis—neither is a functional assay of fertilization competence.
- **Technology: RRBS** under-samples **LINE-1** and intergenic CpG-poor regions; repeat-aware alignments are essential. Orthogonal **long-read** methylomics and **single-cell multi-omics** would refine locus-specific inferences.
- **Causality:** Cross-sectional design cannot establish causal direction; longitudinal conception cohorts will be decisive.

Future Directions

1. **Replication and expansion:** Multi-center replication with larger, age-balanced cohorts; inclusion of **cryptorchidism/hypospadias histories** to position findings within the TDS spectrum.
2. **Orthogonal validation: Amplicon bisulfite** and **Nanopore** methyl-calling at **sentinel Alu-EREs** across sperm fractions and in **testicular germ-cell** subtypes.

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3. **Functional tests:** CRISPR-dCas9-DNMT/TET editing to modulate **Alu-ERE methylation** in **spermatid-like cells** and read out effects on **chromatin compaction**, **DNA damage**, and **apoptosis**.
4. **Exposure biology:** In vitro **human gonadal organoids**/PGC-like cells exposed to **ER agonists/antagonists** (and phthalates/BPA) to model **locus-specific methylation changes** at repeats.
5. **Clinical translation:** Develop a **composite risk score** (AGD + sperm subfraction proportions + repeat methylation signature) for **idiopathic infertility** counseling and **ART pathway selection**.

Conclusions

Men who present with **shortened AGD** and infertility frequently reside within the **TDS continuum**, reflecting **disrupted fetal testis programming**. A mechanistically coherent, testable model—supported by clinical methylome profiling—is that **estrogen-responsive Alu elements** in sperm carry **persistent methylation alterations** in such men, particularly within **chromatin-immature** and **apoptosis-prone** sperm subpopulations. This model integrates **fetal endocrine disruption**, **PGC epigenetic reprogramming**, and **adult sperm function**, offering a route to **molecular biomarkers** and **preventive strategies**. Translational next steps include **multicenter validation**, **orthogonal epigenomics**, and **functional perturbation** of **Alu-ERE methylation** to establish causality and clinical utility.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Hypothalamus–pituitary–gonadal axis in cryptorchid boys

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Abstract

Cryptorchidism affects ~2–5% of male infants at birth and ~1% at 1 year and is strongly associated with later subfertility and an increased risk of testicular cancer. Beyond mechanical maldescent, a growing body of data suggests that in a sizeable subset of boys, cryptorchidism reflects a disturbance of the hypothalamus–pituitary–gonadal (HPG) axis during fetal life and especially during *mini-puberty*—the postnatal surge of gonadotropins and testosterone that orchestrates Sertoli and Leydig cell maturation and the transformation of fetal gonocytes into adult dark (Ad) spermatogonia, the stem cell pool for adult spermatogenesis. This article synthesizes evidence on HPG function in cryptorchid boys, integrates prospective histology–endocrine correlations, and evaluates how timing and modality of therapy (orchiopexy alone versus combined with hormone/gonadotropin therapy) influence germ-cell outcomes and adult fertility. Drawing on published studies and the author's clinical observations and cohort analyses, we argue that: (1) unilateral cryptorchidism often behaves as a bilateral disease at the tissue level; (2) routine serum hormones after mini-puberty have limited diagnostic power for individual risk stratification unless interpreted against histology; (3) the most consistent endocrine signal of high infertility risk is relative LH insufficiency during mini-puberty, not a primary FSH defect; (4) orchiopexy improves anatomic position but does not restore a previously abrogated mini-puberty; and (5) targeted endocrine rescue—particularly GnRH/gonadotropin-based induction of mini-puberty—can normalize Ad spermatogonia formation and improve long-term semen quality in selected patients. Practical implications include earlier evaluation focused on risk stratification (not merely scheduling surgery), judicious use of testicular biopsy in expert hands, and clinical trials of mini-puberty induction guided by robust biomarkers and safety endpoints.

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Introduction

Cryptorchidism (undescended testis, UDT) is a common pediatric condition with heterogeneous etiologies and phenotypes. While surgical relocation (orchiopexy) before 12 months has become standard to reduce risks of impaired spermatogenesis and malignancy, the persistent observation of suboptimal adult semen parameters in many men treated in childhood, even after timely surgery, indicates that maldescent is frequently accompanied by primary testicular dysgenesis and/or disturbances of the HPG axis in critical developmental windows [1].

A central theme of contemporary andrology is *mini-puberty*: a transient activation of the HPG axis from ~2–4 weeks to ~3–6 months postnatally, when pulsatile GnRH drives pituitary LH and FSH secretion, raising testosterone in boys and stimulating Sertoli cell products (AMH, inhibin B). Mini-puberty calibrates reproductive "set-points" and promotes transformation of fetal gonocytes into Ad spermatogonia—the stem cells essential for future spermatogenesis [2, 3, 4, 5]. Mini-puberty metrics—most notably serum testosterone near 3 months—predict adult total sperm count in population cohorts, underscoring causality between early HPG activity and later fertility [6, 1].

For cryptorchid boys, two (nonexclusive) mechanistic frames have been debated for decades: (i) predominantly testicular dysgenesis (primary germ cell/Sertoli cell defects), and (ii) a neuroendocrine endophenotype featuring relative gonadotropin (especially LH) insufficiency during mini-puberty. Clinical series, endocrine studies, and biopsy-linked cohorts now suggest both patterns exist; crucially, the endocrine signature and tissue phenotype co-segregate and guide treatment response [1,4].

This paper reviews the biology of the HPG axis relevant to cryptorchidism, examines evidence for endocrine abnormalities in cryptorchid boys (with and without biopsy stratification), and discusses therapeutic implications, integrating data from randomized and longitudinal studies of GnRH/gonadotropin therapy and orchiopexy. Observations, figures, and interpretations are aligned with remarks delivered at the 5th International Andrology Symposium, *Cryptorchidism: Molecular Biology Meets Endocrinology and Surgery*, Valletta, Malta, 26–27 September 2025 (transcript excerpts cited where relevant) and with the author's published cohort work [7].

Developmental physiology: the HPG axis and mini-puberty

Fetal and early postnatal windows

Testicular descent involves hormonally orchestrated transabdominal and inguinoscrotal phases driven by INSL3, gonadotropins and androgens with key roles for the gubernaculum and epididymis, which guides and precedes the testis into the scrotum [8,9]. In parallel, intra-testicular events include migration and maturation of gonocytes toward the basement membrane and their transformation into Ad spermatogonia, a step tightly coupled to androgen and FSH/inhibin B signaling during mini-puberty [4,5,10].

Mini-puberty in boys is characterized by elevated LH (stimulating testosterone production) and FSH (driving Sertoli cell proliferation and inhibin B). Large normative and disorder-specific

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datasets, including systematic reviews, confirm the existence and clinical utility of this window; its disruption in congenital hypogonadotropic hypogonadism (CHH) underscores causality, and recent consensus statements endorse early gonadotropin therapy for CHH to induce mini-puberty [10,11].

Mini-puberty and later fertility

A landmark Nordic birth cohort showed that higher serum testosterone at ~3 months predicts higher total sperm count in adulthood, even after adjustment for confounders [6]. This finding substantiates mini-puberty as a *programmable* set-point with lifelong impact. Reviews from 2020 to 2024 and 2025 updates reiterate that biomarkers in infancy correlate with pubertal/adult reproductive parameters [2,3,4,12].

Cryptorchidism as an HPG disorder: what do hormones really show?

Population studies vs biopsy-linked cohorts

In unselected cryptorchid cohorts during mini-puberty, several studies reported higher FSH (± higher LH) with lower inhibin B compared with controls, signaling Sertoli cell dysfunction; others found subtler or no differences—discrepancies that reflect phenotypic heterogeneity, small sample sizes, and absence of histological stratification [2,5,13].

When endocrine data are *paired with biopsy*, a clearer pattern emerges testes with severe germ cell depletion and Sertoli-cell–only (SCO) histology often coincide with **relative LH insufficiency** [7, 14]. In our prospective study of cryptorchid boys, the high-risk (adverse histology) group exhibited *lower basal LH* despite similar ages, supporting a central (HPG) insufficiency endotype [7]. The same signal has been observed in independent Danish cohorts when boys are separated by biopsy-defined good vs bad histology [15]. These data help reconcile why non-biopsy cohorts can look "normal" or only subtly abnormal: risk is concentrated in a subset with specific histology and LH dynamics.

Is unilateral cryptorchidism a "bilateral disease"?

Multiple clinical and histological series report pathology in the contralateral scrotal testis of boys with unilateral UDT, from reduced germ cells per tubule to impaired Ad spermatogonia counts, implying systemic or central influences rather than purely local maldescent [1,16]. Experimental models and human histology underscore that ostensibly "normal" contralateral testes often harbor maturational defects. Early classic pathology and more recent analyses concordantly indicate bilateral impact, indicating suboptimal minipuberty [16].

Why routine hormones after 12–18 months disappoint

By ~12–18 months, LH has largely returned to quiescence; group differences narrow and clinical assays may lack sensitivity at low concentrations. Thus, single time-point hormones beyond mini-puberty are poor *individual* classifiers unless paired with histology or dynamic tests. This explains why ROC curves based on routine LH/FSH/inhibin B around 15 months are weak in unselected cohorts [7].

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Histology as the ground truth: Ad spermatogonia and germ-cell counts

The number of Ad spermatogonia per tubule (AdS/T) and the germ cells/tubule (G/T) are powerful predictors of adult semen quality. Pioneering and subsequent work showed that failure of Ad transformation during mini-puberty is tightly associated with oligozoospermia/azoospermia in adulthood—even when orchiopexy was timely and technically successful [1,4,6,10]. A 2019 series specifically assessed unilateral UDT and developed histological risk criteria for azoospermia; again, tissue readouts outperformed routine hormones for risk stratification [1,16].

Surgery helps—but cannot "rewind" a missed mini-puberty

Orchiopexy outcomes

Earlier orchiopexy (ideally before 12 months) improves testicular growth and some surrogate fertility markers, but *does not* fully normalize germ-cell development if mini-puberty was defective beforehand. Post-orchiopexy histology months to years later often shows no histological rescue if the Ad pool was not established, aligning with the concept that surgery corrects position but not prior endocrine programming [1,4,12].

Epididymal anomalies and obstruction: an overlooked axis

Epididymal and vaso-epididymal anomalies, present in ~20% of UDTs, are frequently associated with sperm outflow obstruction and can limit fertility despite otherwise favorable testicular histology [17,18,19]. The epididymis and gubernaculum play leading roles in descent; embryologic and comparative studies (mouse–opossum) and focused reviews emphasize that the epididymis often "leads" and the testis follow—an anatomic nuance with practical implications for surgery and counseling [9,20].

Endocrine rescue of mini-puberty: evidence for GnRH/gonadotropins

If the core defect in a subset is failure of mini-puberty, then *inducing* or *rescuing* it should improve germ-cell maturation. Small randomized and prospective studies of GnRH agonist (LH-RHa) in bilateral cryptorchidism demonstrate restoration of mini-puberty signals and, crucially, **completion of gonocyte→Ad transformation** in treated testes versus surgery alone [21, 22]. Molecular analyses suggest that GnRHa modulates transcriptional programs underlying Sertoli and germ cell maturation (e.g., NHLH2-linked axes) [22].

Longer-term, a 16-year longitudinal cohort comparing immediate versus delayed versus combined (hormone+surgery) therapy found **better adult semen quality** with prolonged hormonal therapy irrespective of whether descent was ultimately surgical, supporting a role for endocrine programming beyond mechanical descent [3,23]. Contemporary systematic reviews in CHH and in mini-puberty induction reinforce efficacy of gonadotropins/GnRH in appropriate indications while noting heterogeneity and the need for standardized protocols and safety data [11,24].

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Reconciling apparently conflicting endocrine studies

Reports that "there is no difference" in mini-puberty hormones in cryptorchid infants typically suffer from: (1) absence of biopsy stratification; (2) inclusion of mixed phenotypes (congenital, acquired/ascending); (3) cross-sectional timing outside peak mini-puberty; (4) assay sensitivity limits at very low LH after 6–9 months; and (5) small sample sizes. When these biases are addressed, a consistent picture emerges:

- **During classic mini-puberty (≈2–4 months):** cryptorchid infants as a group often have higher FSH and lower inhibin B; LH/testosterone defects are subtler and cohort-dependent [2, 3].
- **In histology-defined high-risk cases after mini-puberty: lower LH** (relative gonadotropin insufficiency) is the discriminant signal; FSH is frequently non-discriminatory at the individual level [7,15].
- **After mini-puberty (~>9–12 months):** routine hormones poorly separate risk strata; consider biopsy and/or dynamic testing [7].

Clinical decision-making: a practical algorithm

1. **Early referral focused on *evaluation*, not automatic surgery.** Aim to assess by 2–4 months to exploit the mini-puberty window for endocrine phenotyping (LH, FSH, testosterone, inhibin B), with attention to assay sensitivity and age-appropriate reference intervals [2,3,5,13].
2. **Plan orchiopexy by 6–12 months but acknowledge limits.** Early surgery improves many parameters yet does not rescue missed mini-puberty; technique must be tailored, with particular attention to epididymal/vas anomalies that may predict obstructive components of future infertility [4,19,25,26,27]. Consider *targeted* testicular biopsy by expert surgeons to quantify Ad S/T and G/T. Histology is the strongest predictor of infertility risk and therapy responsiveness [1,7,10].
3. **Consider endocrine induction of mini-puberty in selected cases.** For infants with evidence of mini-puberty failure (low LH/testosterone at 2–4 months, severe histology, bilateral UDT, micropenis, CHH suspicion), discuss GnRH/gonadotropin therapy using protocols supported by trials and systematic reviews; ideally within clinical trials or registries to ensure standardized dosing, monitoring, and long-term follow-up [11,16,21].
4. **Long-term follow-up into adolescence and adulthood.** Track testicular volume, semen parameters (when appropriate), and endocrine status. Even unilateral cases warrant counseling on bilateral disease risk at the tissue level [1,12].

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Special topics

Acquired/ascending testes vs. congenital UDT

Phenotypes differ acquired cryptorchidism may have later onset and distinct endocrine features. Many negative endocrine studies included patients with ascending testes, diluting signals of genuine mini-puberty failure present in congenital cases.

The epididymis and the "guidance" hypothesis

Surgical observations and embryology argue that the epididymis often anchors the descent path: the gubernaculum attaches to the caudal epididymis and expands the inguinal canal; the testis "follows" down this tract [9,21]. Recognizing and documenting epididymal disjunction or atresia during orchiopexy is crucial for prognostication of obstructive infertility.

Safety of early surgery

Operating at 6 months requires experienced teams; some series suggest higher complication rates in very small infants, though contemporary pediatric centers report excellent outcomes. The benefit–risk calculus should integrate endocrine status, testis position, anesthesia safety, and institutional expertise [25].

Synthesis and working model

Model A (HPG-intact dysgenesis): Primary testicular dysgenesis with intact HPG axis yields elevated FSH (due to low inhibin B), normal/near-normal LH/testosterone during mini-puberty, poor histology (SCO/fibrosis), and limited response to endocrine rescue. Surgery repositions but cannot create Ad spermatogonia de novo.

Model B (HPG-axis insufficiency during mini-puberty): Relative LH deficiency blunts Leydig activation, reduces intratesticular testosterone, and **blocks the gonocyte→Ad transformation**. Histology shows low Ad, S/T with otherwise salvageable architecture. Here, **GnRH/gonadotropin therapy** during the ‚window can normalize Ad, S/T and later semen counts, while surgery alone improves position but not the stem cell pool [1,21,22,23].

Reality: Many boys sit along a spectrum; unilateral UDT often conceals bilateral tissue vulnerability. This heterogeneity explains disparate results in unstratified studies and highlights the need for individualized, biology-informed care.

Implications for the next decade

1. **Standardize mini-puberty testing.** Age-specific references, ultrasensitive LH assays, and harmonized sampling at ~10–14 weeks will sharpen phenotyping [3,5].
2. **Biopsy-guided trials.** Randomized studies of mini-puberty induction should require baseline histology (Ad, S/T, G/T) and report tissue and molecular endpoints alongside clinical descent and semen outcomes [21,22,28].

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3. **Endpoints that matter.** Adult total sperm count is the meaningful endpoint, as validated by population cohorts linking infant T to adult semen quality [1,4,6].
4. **Epididymal/vas mapping.** Routine intraoperative documentation (photography, standardized anomaly scoring) will inform obstruction-related infertility risk and direct adolescent/adult management [18,19].
5. **Translational biology.** Single-cell and spatial transcriptomics of infant testes pre- and post-GnRHa could decode the gene programs governing Ad formation and identify novel druggable targets [28].

Conclusions

Cryptorchidism is not merely a problem of malposition. For many boys it reflects a *developmental endocrinopathy* in which mini-puberty is blunted—most conspicuously through **relative LH insufficiency**—resulting in failure to establish the Ad spermatogonial pool. Orchiopexy is necessary but not sufficient to fix an early endocrine miss. Biopsy-informed care and *timely endocrine induction of mini-puberty* can rescue germ-cell development and improve adult semen outcomes in selected cases. The field's task is to move from one-size-fits-all surgery to **biology-tailored therapy** that begins with early evaluation and ends with proven fertility gains.$

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication; Not applicable

c) Availability of data and supporting material; Not applicable

d) Competing interests Author/s declare that they have no competing interests

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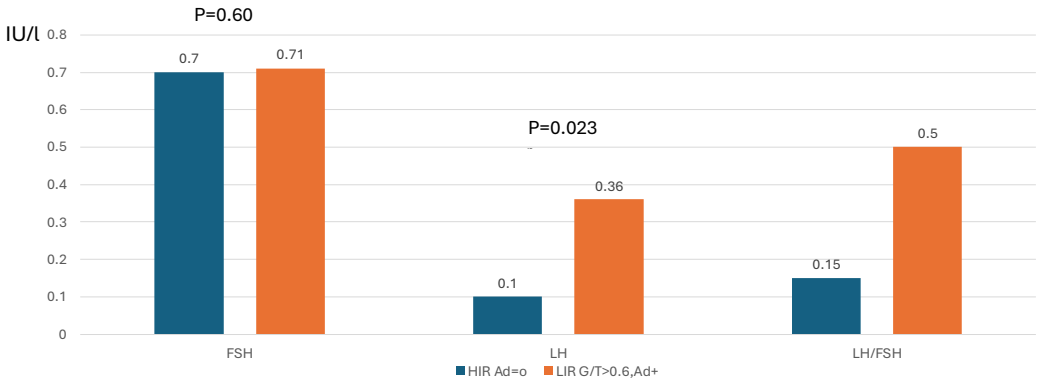

Figure 1. Hormone levels in testicular samples from HIR/LIR patients. Median FSH and LH levels and LH/FSH ratios are plotted against 21 samples from HIR and 18 samples from LIR cryptorchid boys. Wilcoxon-Mann-Whitney test was performed. P values are given at the top. HIR: high infertility risk; LIR: low infertility risk; FSH: follicle-stimulating hormone; LH: luteinizing Hormone [15]

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Histopathology of cryptorchid testis

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Abstract

Cryptorchidism—testicular maldescent—affects 2–5% of newborn boys and ~1% at 12 months, and is linked to infertility and increased testicular germ cell tumor risk in adulthood. Histopathology provides the closest “ground truth” for prognosis, because tissue read-outs (germ cell number per tubule, presence of adult dark [Ad] spermatogonia, Sertoli-cell–only [SCO] patterns, interstitial changes) integrate upstream endocrine and paracrine events and the thermal microenvironment. This proceedings paper reviews the classical and modern histopathology of cryptorchid testes (as seen on light microscopy), highlights the methodological importance of optimized fixation and semithin resin sections for identifying Ad spermatogonia, synthesizes how biopsy metrics stratify fertility risk, and summarizes the pathology of germ cell neoplasia in situ (GCNIS) in this setting. We also integrate cohort data from Vilnius–Basel collaborations and others showing that unilateral cryptorchidism often behaves as a bilateral disease at the tissue level and that the presence or absence of Ad spermatogonia (and related indices) outperforms routine serum hormones for individual fertility prediction. Finally, we propose a practical reporting template for pediatric testicular biopsies taken at orchiopexy that preserves clinical decision-making value (fertility risk, need for endocrine induction of mini-puberty, timing of surveillance for malignancy) while respecting tissue limits and safety.

Key words Cryptorchidism histology ad spermatogonia mini puberty

Résumé

La cryptorchidie—maldescente testiculaire—touche 2 à 5 % des nouveau-nés de sexe masculin et environ 1 % à 12 mois, et s’associe à une infertilité accrue et à un risque majoré de tumeur germinale testiculaire à l’âge adulte. L’histopathologie constitue l’indicateur pronostique le plus fiable, car les paramètres tissulaires (nombre de cellules germinales par tube, présence de spermatogonies adultes sombres [Ad], aspects de type Sertoli-cell-only [SCO], modifications interstitielles) reflètent l’ensemble des événements endocrines, paracrines et thermiques antérieurs.

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Cet article de synthèse passe en revue l'histopathologie classique et contemporaine des testicules cryptorchides en microscopie optique, souligne l'importance d'une fixation optimisée et des coupes semi-fines en résine pour l'identification des spermatogonies Ad, et montre comment les indicateurs histologiques permettent de stratifier le risque de fertilité. La pathologie de la néoplasie germinale in situ (GCNIS) dans ce contexte est également résumée. Nous intégrons les données de cohortes, notamment des collaborations Vilnius–Bâle, indiquant que la cryptorchidie unilatérale présente fréquemment un phénotype bilatéral au niveau tissulaire et que la présence ou l'absence de spermatogonies Ad constitue un meilleur prédicteur individuel de fertilité que les hormones sériques usuelles. Enfin, nous proposons un modèle pratique de compte rendu anatomopathologique pour les biopsies testiculaires pédiatriques réalisées lors d'une orchidopexie, afin d'optimiser la valeur décisionnelle (risque de fertilité, indication d'induction endocrine de la mini-puberté, stratégies de surveillance oncologique) tout en respectant les contraintes tissulaires et la sécurité.

Mots-clés: Cryptorchidie, histologie, spermatogonies Ad, mini-puberté

Introduction

Why scrutinize the histology of cryptorchid testes? First, because adult fertility correlates strongly with whether the infant testis establishes a pool of Ad spermatogonia during mini-puberty, a developmental window in which luteinizing hormone (LH) and testosterone peak and Sertoli cell function is calibrated. Failure of gonocyte→Ad transformation yields a depleted stem-cell reservoir and predicts poor semen parameters decades later—even if orchiopexy is anatomically successful. Histology captures this failure directly. Large clinicopathologic series confirm that quantifying germ cells per tubule and documenting Ad spermatogonia during childhood stratifies azoospermia risk and informs therapy. [1–4]

Second, histology remains the only practical way to diagnose GCNIS (the universally accepted precursor to most post-pubertal testicular germ cell tumors) in equivocal settings, and to document ancillary risk features (multinucleated spermatogonia, tubular atrophy/fibrosis). Consensus taxonomies and ISUP/WHO 2016 nomenclature standardize these entities and their reporting. [5–7]

Finally, histology is a bridge between basic and clinical science. It is where the "HPG-axis hypothesis" and the "primary testicular dysgenesis hypothesis" leave footprints (Leydig cell size/number, Sertoli cell maturation, tubular architecture, interstitium), and where the consequence of temperature and timing is recorded in resin and glass. [2,3,8]

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Methods matter: obtaining interpretable pediatric testicular biopsies

Fixation, processing, and sectioning

Routine 10% neutral buffered formalin (NBF) paraffin processing, while ubiquitous, introduces shrinkage and sloughing artifacts that obscure germ cell cytology in infant testes; it often prevents reliable discrimination between Ad and Ap spermatogonia. [9] For *fertility-oriented* pediatric testis biopsies, **semithin (0.5–1 µm) sections of epoxy-resin–embedded tissue stained with toluidine blue** preserve subcellular detail and allow confident identification of spermatogonial subclasses. This technique, refined in andrology and NOA (non-obstructive azoospermia) practice, is considered the *most suitable* approach for evaluating spermatogenesis histologically. [10-13]

Key technical points:

- **Immediate fixation** in glutaraldehyde-based or mixed fixatives suitable for resin infiltration mitigates artifactual cell loss. [10-12]
- **Semithin epoxy sections** maximize nuclear/cytoplasmic contrast; **toluidine blue** metachromasia highlights chromatin patterns critical for classifying Ad vs Ap spermatogonia. [10-13]
- **Sampling**: Bilateral biopsies during orchiopexy increase diagnostic yield and capture asymmetry; even unilateral cryptorchidism can have contralateral abnormalities. [1,10,14]
- **Quantification standards**: Count total seminiferous tubules (TST), tubules containing spermatogonia (TCS), **germ cells per tubule (G/T)**, and **Ad spermatogonia per tubule (AdS/T)**; calculate a **Fertility Index (FI)** (spermatogonia-positive tubules ÷ total tubules), as popularized in classic and contemporary series. [4,15,16]

What the microscope shows in cryptorchid testes

The “triad” of cell lineages: germ cells, Sertoli cells, Leydig cells

Germ cells. The signature lesion is *depletion* of germ cells with failure of gonocyte→Ad transformation. Under light microscopy (semithin, toluidine blue), **Ad spermatogonia** are recognized by their dense, spherical nuclei with a characteristic **perinuclear rarefaction halo** and clumped chromatin—features that are difficult to appreciate in paraffin H&E but crisp in resin sections. Their **absence** defines a **high infertility risk (HIR)** phenotype; **presence** (≥1 Ad per ≥1 tubule) indicates **low infertility risk (LIR)**—a dichotomy repeatedly validated against semen outcomes. [2–4,10,15]

Sertoli cells. Cryptorchid testes often show **developmental arrest** of Sertoli cells: tall cells with immature cytoplasm, persistent fetal markers, and loss of proper polarity; SCO patterns may appear in the most severe cases. [1–3,5]

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Leydig cells. Contrary to the lay expectation of Leydig hyperplasia, pediatric cryptorchid testes frequently exhibit **Leydig cell atrophy** and vacuolization on semithin sections—an anatomic correlate of impaired LH-Leydig signaling during mini-puberty in a subset of boys. [1–3,10,14]

Tubulointerstitium. Early **peritubular fibrosis**, basement membrane thickening, and reduced tubular diameter track with germ cell loss. Interstitial edema can be seen in acute torsion/iatrogenic injury but is not a hallmark of ordinary maldescent. [1–3,5]

Spectrum of patterns and their prognostic weight

- **Low infertility risk (LIR)**: many tubules with spermatogonia; **Ad present** in at least one tubule; modest reduction of G/T; minimal peritubular fibrosis; Sertoli cells maturing. Long-term cohorts show much better semen counts from adolescence onward. [1–4,16]
- **High infertility risk (HIR)**: **Ad absent**; many tubules empty or with rare Ap only; marked reduction of G/T; peritubular fibrosis prominent; Leydig cell atrophy common. Without endocrine rescue, adult semen parameters are often severely compromised even after timely orchiopexy. [1–4,10,16]

Sertoli-cell–only (SCO) / extreme HIR: essentially no recognizable spermatogonia; severe tubular atrophy/fibrosis. [1–3,5]

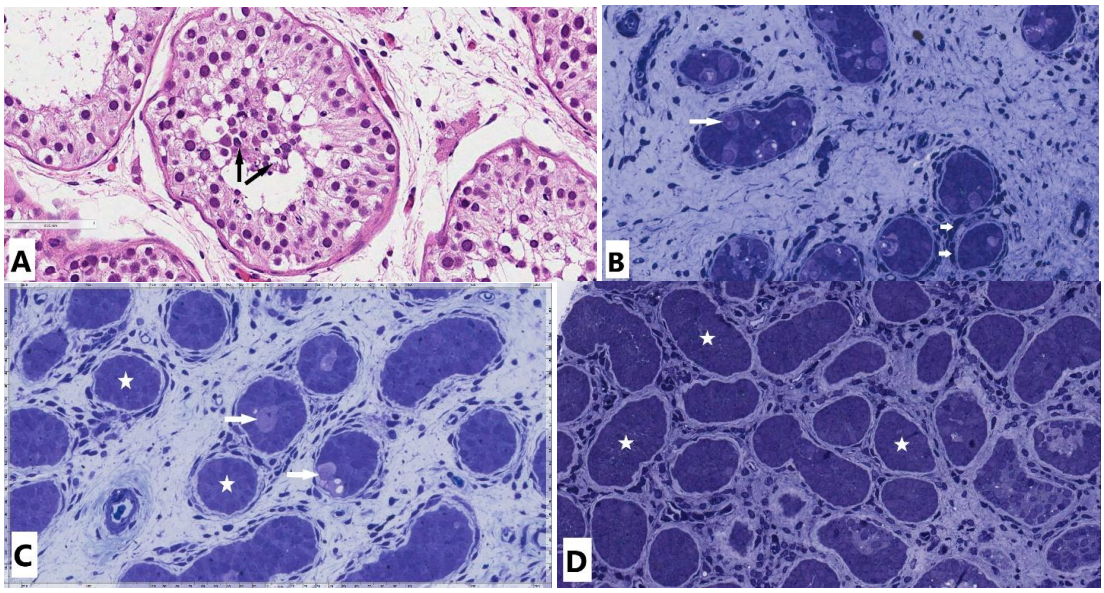

Fig.A. Marked shrinkage artifacts are evident within the germinal epithelium. Proper identification of Ad spermatogonia is barely possible. Formalin fixed, Paraffin embedded tissue. H&E staining, 40x. Fig.B. Low Infertility Risk. All tubular cross-sections have spermatogonia, some - Ad spermatogonia (long arrow); broad interstitium with several atrophic Leydig cells (short arrows). Toluidine staining, 40x. Fig.C. High Infertility Risk. Diffuse decrease of tubular density. Severe germ cell depletion (arrows). No Ad spermatogonia. Most

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of the tubules contain only Sertoli cells (5-star). Toluidine staining, 40x. Fig.D. High infertility risk. All tubules contain only Sertoli cells (5-stars), with no spermatogonia observed. Toluidine staining, 40x.

-

The **contralateral "normal" testis** in unilateral cryptorchidism frequently shows subclinical abnormalities (lower G/T, rare Ad, subtle SC dysmaturation) consistent with a bilateral disease model—one reason unilateral cases can still have reduced adult semen quality. [1,14]

Counting what matters: Ad spermatogonia, G/T, and the Fertility Index

Across classic and modern studies, **Ad presence in infancy/early childhood** is the strongest histologic predictor of adult sperm output. The **Fertility Index** (fraction of tubules containing any spermatogonia) complements **G/T** and **AdS/T** to capture both *distribution* and *depth* of the germ-cell deficit. A meta-analytic theme is consistent: **Ad+** cryptorchid boys fare far better than **Ad−** peers, even when both receive early orchiopexy. [15–17]

These indices also serve as **biomarkers of response** to endocrine therapy. In randomized/controlled settings, **GnRH agonist (LH-RHa)** or gonadotropin regimens administered around mini-puberty increased **G/T** and converted some **Ad−** testes to **Ad+**, whereas surgery alone did not. [2,3,18,19]

Histology and mechanisms: beyond temperature

Histology also speaks to mechanism. The **HIR** pattern (Ad−, Leydig atrophy, preserved FSH-Sertoli drives with failing LH–Leydig output) aligns with a **mini-puberty insufficiency** endotype. Tissue-level rescue after LH-RHa, including transcriptomic reprogramming of chromatin modifiers (e.g., **PRDM** family HMTs), has been documented in Vilnius–Basel cohorts, providing molecular plausibility for the histologic changes we see. [2,3]

A recent work further argues that **temperature alone** is insufficient to explain the failure of Ad formation: with LH-RHa, differentiation to **Ad** occurred **despite** cryptorchid position, indicating that endocrine milieu can override thermal disadvantage at this developmental stage. [3]

Pathology of malignancy risk in cryptorchidism

GCNIS: names, morphology, and prevalence

Formerly "CIS/IGCNU/TIN," **germ cell neoplasia in situ (GCNIS)** is the WHO-endorsed precursor of most post-pubertal TGCTs. Histologically, large atypical germ cells with

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abundant pale cytoplasm and prominent nucleoli line the basal compartment of seminiferous tubules; immunoprofiles include PLAP, OCT3/4, and c-KIT positivity. [5-7]

Prevalence in cryptorchid adult testes is variably reported from ~**1.7% to 8%**, with several biopsy series centering around **~3%**, acknowledging selection and sampling biases. [20-23] Importantly, **~50%** of men with biopsy-proven GCNIS will develop invasive cancer within ~5 years without treatment, which underpins surveillance and management recommendations. [20,21]

Does orchiopexy abolish cancer risk? No. Early orchiopexy appears to **reduce** relative risk compared with post-pubertal repair, but **does not eliminate** it; hence lifelong awareness and self-examination are recommended. [24–26]

Other malignancy-adjacent histologies

- **Multinucleated spermatogonia** (MNG) can be observed in dysgenetic tubules; they signal disturbed spermatogonial kinetics but are not by themselves premalignant. [1,5]
- **Severe tubular atrophy and fibrosis** reduce the number of "at-risk" intratubular niches but co-exist with GCNIS when present. [5–7]

Putting it together in practice: a pathology reporting blueprint

When a **pediatric** testicular biopsy is performed at orchiopexy (in experienced centers, with prior counseling), the pathology report should prioritize **fertility risk** and **malignancy surveillance** implications while documenting background features that hint at mechanism.

Suggested minimum dataset:

1. **Processing**: state resin-semithin vs paraffin; stain(s) used; block sufficiency. [10–12]
2. **Quantification** (supply age in months to aid interpretation):
    - **TST**, **TCS**, **G/T (median)**, **AdS/T**; **Fertility Index**. [15–17]
    - Tubular diameter (µm), peritubular fibrosis (semiquantitative). [1–3,5]
3. **Lineage notes**: Sertoli cell maturation (immature vs maturing vs SCO); Leydig cell morphology (atrophy, vacuolization). [1–3,10]
4. **Risk call**: **Low infertility risk** (Ad present) vs **High infertility risk** (Ad absent). [1–4,15–17]
5. **GCNIS**: present/absent; if present, immunostains used. [5–7,20–21]
6. **Comment**: tie to clinical context (unilateral/bilateral, intra-abdominal vs inguinal, age), suggest implications—e.g., "Findings consistent with high infertility risk (Ad−); consider endocrine induction of mini-puberty within trial/registry," or "No GCNIS identified; risk not eliminated; adhere to long-term surveillance guidance." [1–4,18–19,24-26]

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How histology informs management

- **Surgery is necessary but not sufficient.** Orchiopexy relocates the testis and eases surveillance but does **not** rebuild a missing Ad pool. Histology can show whether surgery is likely to "ride the wave" of intact biology (Ad+) or needs **endocrine rescue** (Ad−). [1–4,18–19]
- **When Ad is absent** (HIR), evidence supports considering **GnRH agonist** (or gonadotropin) protocols to "induce mini-puberty," with tissue-level and later semen benefits in selected cohorts. [2,3,18,19]
- **When GCNIS is found**, urologic-oncologic algorithms apply (radiation in selected cases vs surveillance vs orchiectomy, depending on age, fertility plans, and contralateral status). [5–7,20–21,24–26]

Limitations and future directions

Sampling bias (tiny biopsies in a mosaic organ), **assay variability** (non-standardized counts), and **processing heterogeneity** (paraffin vs resin) complicate cross-study comparisons. Prospective registries using harmonized semithin protocols, age-matched norms, and blinded central review are needed. On the translational side, **single-cell/spatial transcriptomics** pre- and post-endocrine rescue can connect histologic rescue (Ad appearance) to gene-program activation (e.g., chromatin remodelers such as **PRDM** HMTs). [2,3]

Closing remarks (linking back to the talk)

Pathology can deliver clinically decisive information **if** we give it the right tissue, processed the right way, and if we ask the right questions. In cryptorchidism, the question is not only "where is the testis?" but "**what is inside it, now, at this age?**"—and what that portends for this child's future fertility and cancer risk. The semithin-resin approach makes **Ad** visible; the counts make risk quantifiable; and the vocabulary (GCNIS) makes risk shareable. That—together with multidisciplinary decision-making—is the core message from our Vilnius practice and the European collaborations we are grateful to contribute to. (Conference transcript)

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Negative effect of hormonal treatment on prepubertal testis

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Abstract

Whether exogenous hormones should be used in boys with cryptorchidism (undescended testis, UDT) remains one of the most debated questions in paediatric andrology and urology. European/American practice guidelines no longer recommend hormonal therapy to induce descent, citing low success rates and uncertain long-term benefit, whereas a European panel has a weak recommendation of gonadotropin-releasing hormone (GnRH) analogues as adjuvant therapy to potentially preserve fertility indices in selected boys—especially bilateral cases—after orchidopexy.

This paper reviews histological, endocrine, and clinical evidence for adverse effects of hormonal treatment administered in the prepubertal years, with focus on hCG (human chorionic gonadotropin) and GnRH (LHRH) regimens used historically for “therapeutic descent.” It synthesizes data from Danish and Nordic cohorts (including our own), randomized/controlled studies, and translational work on germ cell apoptosis and suppression of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Key findings are:

- In boys aged 1–3 years, unsuccessful courses of GnRH or hCG reduced the number of spermatogonia per tubule at the time of orchiopexy compared with surgery alone (primary histological endpoint).
- HCG can acutely raise testosterone to adult levels in prepubertal boys while suppressing pituitary FSH/LH, disturbing the endocrine milieu thought to support germ cell proliferation and transformation; this pituitary suppression after hCG has been documented in Danish cryptorchid cohorts.
- Multiple human studies show increased germ cell apoptosis shortly after hCG exposure in cryptorchid and contralateral/scrotal testes; in long-term follow-up, prior hCG has been linked to impaired adult reproductive function in some series.
- Dose and age window matter: high-dose regimens used historically (e.g., hCG 100 IU/kg (maximum 1,500 IU) twice weekly × 3 weeks; or daily nasal LHRH 1.2 mg/day for 28 days in 2 series) in boys under ~4–6 years appear most likely to show negative

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histological signals; lower adjuvant GnRH doses after surgery in carefully selected infants remain under investigation.

Taken together, available evidence supports the American, Nordic and European consensus position that routine hormonal treatment for cryptorchidism is not recommended—given poor efficacy for descent and possible adverse effects on spermatogenesis—while acknowledging that post-orchiopexy, low-dose adjuvant GnRH analogues may be studied for patients with biopsy-defined reduced germ cell counts and germ cell transformation, and endocrine evaluation revealing insufficient genuine gonadotropin stimulation, under robust protocols.

Key words: Cryptorchidism, hormonal side effects, histology

Résumé

L'utilisation d'hormonothérapie chez les garçons cryptorchides demeure l'un des sujets les plus controversés en andrologie et urologie pédiatriques. Les recommandations européennes et américaines ne préconisent plus l'hormonothérapie pour induire la descente, en raison de son efficacité limitée et de l'absence de bénéfice durable démontré. Un panel européen propose toutefois, avec un faible niveau de recommandation, l'emploi d'analogues de la GnRH comme traitement adjuvant après orchidopexie afin de préserver, chez des garçons sélectionnés — notamment en cas de cryptorchidie bilatérale — certains indices de fertilité.

Cet article analyse les données histologiques, endocriniennes et cliniques relatives aux effets indésirables des traitements hormonaux prépubertaires, en particulier les schémas à base d'hCG et de GnRH historiquement utilisés pour la « descente thérapeutique ». Les résultats issus des cohortes danoises/nordiques, des essais contrôlés et des travaux translationnels sur l'apoptose germinale et la suppression hypophysaire FSH/LH montrent notamment : (i) une réduction du nombre de spermatogonies par tube séminifère après traitements inefficaces chez les garçons de 1 à 3 ans ; (ii) une élévation aiguë de la testostérone à des niveaux adultes sous hCG avec suppression hypophysaire concomitante ; (iii) une augmentation de l'apoptose germinale après hCG dans plusieurs études humaines ; et (iv) des signaux négatifs particulièrement associés aux fortes doses administrées avant 4–6 ans.

Dans l'ensemble, les preuves disponibles soutiennent le consensus américain, nordique et européen selon lequel l'hormonothérapie ne doit pas être utilisée de manière routinière dans la cryptorchidie, compte tenu de sa faible efficacité et de ses effets potentiellement délétères sur la spermatogenèse. Après orchidopexie, l'utilisation adjuvante d'analogues de la GnRH à faible dose pourrait toutefois être envisagée, dans des protocoles contrôlés, chez des enfants présentant une histologie défavorable et un profil endocrinien suggérant une stimulation gonadotrope insuffisante.

Mots clés : Cryptorchidie, effets secondaires hormonaux, histologie

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Introduction

Cryptorchidism affects 2–4% of full-term neonates and ~1–2% at 12 months, with sequelae that include subfertility, infertility and increased risk of testicular germ-cell tumors. The primary treatment is timely orchiopexy (ideally by 6–12 months corrected age, 18 months the latest), a consensus position shared across major guidelines [1–4,].

Where controversies persist is the role of hormonal therapy—either to induce descent or to augment fertility potential. The AUA guideline (2014; validity confirmed 2025) states: "Providers should not use hormonal therapy to induce testicular descent as evidence shows low response rates and lack of evidence for long-term efficacy." [1]. Similarly, the Nordic Consensus concludes that, in general, hormonal treatment is not recommended because of poor immediate results and possible long-term adverse effects on spermatogenesis [2].

By contrast, the EAU/ESPU pocket guideline (2025) (4) and the full EAU/ESPU guideline (2025)(3) have a weak recommendation of offering endocrine treatment (GnRH analogues) in bilateral cases to preserve future fertility potential. The full EAU guideline (2025) emphasizes surgical management and elaborates that identification of specific subgroups of boys with undescended testes who would benefit from using hormones is challenging [3].

This paper is reviewing negative effects observed in prepubertal testes after hormonal therapy, integrating our Danish experience with the broader literature, and clarifying how dose, timing, and endocrine feedback may drive harm.

Background: Why prepubertal testes are vulnerable

Mini-puberty and the germ-cells (first 3 months)

The mini-puberty (peaking around 1–3 months) activates LH, resulting in testosterone production from the Leydig cells, and FSH stimulating the Sertoli cells. These hormonal changes enabling gonocyte → spermatogonia transformation and setting up the Ad (adult dark) spermatogonial stem-cell pool. Disruption of this period (by congenital hypogonadotropic states or testicular dysgenesis) correlates with long-term subfertility or infertility. In 1–4 years there is generally low plateau of the LH/FSH/testosterone levels, but germ-cell proliferation and transformation also take place, and at 4 years of age primary spermatocytes appear [5-7].

The endocrine rationale (and pitfalls) of hCG/GnRH

Earlier clinical practice attempted to mimic hormonal signals: hCG (primarily with LH effect) to stimulate Leydig cells to produce testosterone (and presumed descent via androgen-dependent pathways), or GnRH (with LH and FSH effect) nasal sprays to drive the axis. But in prepubertal boys, hCG can raise testosterone to adult levels and suppress FSH/LH, producing a non-physiological milieu. In a Danish cohort, hCG caused total FSH/LH suppression and very high testosterone [8]; this, resulted in unfavorable germ-cell endpoints when treatment failed to achieve descent [9].

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Evidence of harm: histology, apoptosis, endocrine suppression, and adult outcomes

1) Histology at orchiopexy (primary tissue endpoint)

In a consecutive series of 72 boys aged 1–3 years undergoing biopsies at orchiopexy, those with prior unsuccessful hormonal therapy (GnRH or hCG) had fewer spermatogonia per tubule (S/T) compared with boys who had surgery only; normal S/T values occurred only in the surgery-only group ($p \approx 0.06$), le adding to the conclusion that hormonal treatment may harm germ cells in this age bracket [9].

The clinical protocol that underpinned these observations, daily GnRH 1.2 mg for 28 days, repeat course if no descent, common in Denmark at the time, did not produce descent in boys < 4 years. The boys who were hCG-treated had 100 IU/kg (max 1,500 IU per injection) twice weekly for 3 weeks; six injections, also common in Denmark at the time [9].

Randomized trials elsewhere reported low complete-descent rates with LHRH sprays (e.g., 0.8 mg/day × 28 days improved "some descent" vs placebo without robust complete descent), supporting the guideline stance against hormonal induction [1,2,3,10-12].

2) hCG-associated germ cell apoptosis

Multiple human studies from Finland and elsewhere show a transient spike in germ-cell apoptosis in both cryptorchid and contralateral (scrotal) testes within the first month after hCG exposure [13]. Increased apoptosis after hCG was in adulthood associated with smaller testicular volume of the undescended testis, and elevated FSH, but the sperm count was not reduced [14].

Abrupt androgen rises coupled with pituitary FSH/LH suppression may deprive immature Sertoli–germ cell units of coordinated trophic support; FSH is thought to be permissive for Sertoli proliferation and germ-cell survival in early life. The Danish endocrine studies in cryptorchid boys documented complete suppression of FSH/LH after hCG-courses, with testosterone in the adult range, exactly the pattern that raises concern for desynchrony [8,9].

3) Long-term reproductive outcomes after childhood hormonal therapy

Follow-up cohorts that included boys exposed to pre-orchiopexy hormones versus surgery only report mixed outcomes, complicated by selection bias. Some series found smaller adult testicular volumes in hormonally pretreated patients [14-16], in some no sperm counts were reported [14,15], and others reported similar semen values [14]. At least one study linked prior hCG treatment to worse adult sperm outcomes in subsets, however there was a selection bias as only patients with bilateral cryptorchidism or those with non-palpable testes were offered hormonal treatment [17]. The heterogeneity underscores the risk of confounding but does not negate tissue-level harm signals in the 1–3-year window.

Dose, timing, and formulation: when harm is most likely

"How much is too much?"

Historical Danish protocols used hCG 100 IU/kg twice weekly for 3 weeks (max 1500 IU per injection; six injections) and daily LHRH 1.2 mg/day for 28 days (with repeat cycles if no

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descent) [8,9]. These are high exposures relative to more recent adjuvant GnRH proposals. The negative histology signal in 1–3-year-olds emerged under these doses.

Key principle: the young children (1-4 (6) years with low plateau LH/FSH/testosterone levels) versus older children, and the higher the cumulative hormonal exposure, the greater the concern for germ-cell toxicity and FSH/LH suppression effects.

The adjuvant GnRH question

1. The EAU/ESPU 2025 pocket has a weak recommendation of GnRH analogues as adjuvant to surgery in bilateral cases to preserve fertility indices[4]. Yet the 2025 EAU full guideline stresses that evidence is heterogeneous, and not to use hormones as primary therapy for descent, and that identification of specific subgroups of boys with undescended testes who would benefit from using hormones is challenging [3]. Our own pilot, post-orchiopexy low-dose GnRH-analog trial (Kryptokur® 0.2 mg 2x every second day in 16 weeks) at boys with reduced number of germ cells and no Ad spermatogonia, and despite of this no elevated FHS/LH and therefore signs of a hypogonadotropic state, was stopped for regulatory reasons unrelated to safety, and—while a few boys showed increased number of Ad spermatogonia at re-biopsy—most had no change. We only treated boys with GnRH-analog if they had hypogonadism and insufficient genuine gonadotropin stimulation to avoid pituitary suppression and negative effect on the germ cells [18]. These observations argue for cautious, biopsy- and endocrinological- stratified trials rather than routine use.

Reconciling guidelines and practice

- AUA 2014/2025: Do not use hormonal therapy to induce descent (low response, no durable benefit). Orchiopexy at 6–12 months, 18 months at least [1].
- Nordic Consensus 2007**:** In general, do not use hormonal therapy**—**poor efficacy plus possible adverse effects on spermatogenesis. Orchiopexy at 6–12 months[2].
- EAU/ ESPU 2025: Orchiopexy at 6–12 months, 18 months at least. Hormonal therapy not recommended as primary treatment; panel has a weak recommendation of adjuvant GnRH in cases of bilateral UDT to improve fertility indices [3,4]. The full EAU guideline (2025) elaborates that identification of specific subgroups of boys with undescended testes who would benefit from using hormones is challenging [3].
- Recent reviews echo that most guidelines discourage primary hormonal therapy; if considered for fertility indices, it should be adjuvant, selective, and preferably within trials/registries.

Mechanisms of injury: a working model

1. Axis shock & desynchronization: In the low-hormone prepubertal period, exogenous hCG spikes testosterone, but suppresses FSH/LH (feedback), creating an environment unsupportive of Sertoli-germ co-maturation; apoptosis rises.

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2. Thermal/temperature confounding: Some argue cryptorchid testes are already compromised by temperature; however, the apoptotic surge in both undescended and scrotal testes after hCG implies a direct endocrine effect.
3. Dose dependency & receptor kinetics: Pharmacologic LH receptor stimulation (hCG) in immature Leydig cells may overshoot steroidogenesis while starving Sertoli cells of FSH signals.
4. Window of susceptibility: Infancy to ~4–6 years may be uniquely sensitive; beyond that, pituitary–gonadal dynamics differ.

What the Danish data add

From the 1990s–2000s Danish practice:

- HCG treatment resulted in adult testosterone levels with complete FSH/LH suppression in prepubertal cryptorchid boys [8].
- Histology (age 1–3 years): fewer spermatogonia per tubule after unsuccessful hormonal therapy than after surgery alone [9].
- Clinical observation: daily GnRH 1.2 mg/day for 28 days in two periods did not induce descent in boys < 4 years; all received repeat cycles before proceeding to surgery [10].

These findings underpin our cautionary stance: routine pre-orchiopexy hormonal treatment in this window can be more harmful than helpful**.**

Counterpoints and nuances

Not all studies demonstrated long-term harm. Some follow-ups found smaller testis volumes but similar sperm counts between pretreated and surgery-only groups, suggesting compensation by the contralateral testis[14]. Others reported no sustained increase in apoptosis if surgery occurred > 1 month after hCG [13]. The literature is also complicated by selection bias (hormones given to more severe bilateral/nonpalpable cases) [17]. and variable doses.

Nonetheless, taken in aggregate—with randomized trials showing low descent efficacy and tissue data documenting apoptosis**/**reduced spermatogonia under high-dose regimens in 1–3-year-olds**—**the precautionary interpretation remains justified.

Practical implications for 2025

1. Primary therapy for descent: Do not use hCG or GnRH to induce descent. Refer by 6 months (corrected) and operate before 12–18 months
2. Adjuvant therapy for fertility indices (selected cases): Consideronly supplementary GnRH, within protocols for boys with biopsy-defined risk (e.g., absent Ad spermatogonia, very low G/T) and despite of that blood sample with no elevated FSH/LH, pointing at a hypogonadotropic state—treat after successful orchiopexy, with low-dose GnRH analogues, rigorous monitoring and ethics/authority approvals[18].
3. Avoid high-dose hCG in 1–4-year-olds; be mindful of pituitary suppression and apoptosis data.

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4. Measure what matters: If fertility preservation is the goal, rely on tissue endpoints (Ad spermatogonia presence, G/T), and endocrine parameters (FSH/LH/testosterone, inhibin B).
5. Communicate uncertainty: Families should understand that while early surgery improves prospects, no medical therapy in the prepubertal years has proven long-term fertility benefit that outweighs risks in unselected boys.

Methods appendix (for studies cited)

Where available, we favor randomized/controlled designs for descent efficacy and human tissue studies for germ-cell outcomes with histological analyses and analyses of endocrine dynamics (FSH/LH/testosterone/inhibin B), and rely on prospective sampling around treatment windows. Long-term outcomes are from retrospective cohorts with acknowledged selection biases.

Conclusions

The weight of evidence indicates that prepubertal hormonal therapy—particularly high-dose hCG and course-repeated GnRH regimens historically used to induce descent—can negatively affect the prepubertal testis**,** chiefly by (i) provoking germ-cell apoptosis and (ii) suppressing pituitary FSH/LH at a time when coordinated, low-level trophic support is crucial for establishing the spermatogonial stem-cell pool, transformation of spermatogonia to A dark spermatogonia and to primary spermatocytes. Tissue read-outs at orchidopexy in 1–3-year-olds show fewer spermatogonia per tubule after unsuccessful hormonal therapy than after surgery alone.

In line with AUA, Nordic and European recommendations, routine hormonal therapy to induce descent should be avoided. If hormonal modulation is contemplated post-orchidopexy to rescue fertility potential, it should be restricted to clinical trials with biopsy-based selection (low number of spermatogonia per tubule and no A dark spermatogonia), and simultaneously a hypogonadotropic state (not elevated FSH/LH - despite germ cell hypoplasia), low-GnRH dose regimens, and independent ethical oversight.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Editorial comments

Lacking outcome (spermiograms) Cortes et al. concluded that HCG treatment administered before the age of three years results in severe testicular pathological changes and infertility. Recently, the opposite was reported by Bartoletti et al., who have shown that HCG treatment during the second year of life has no long-term negative side effect on fertility. Importantly, no positive impact from early surgery was found. Thus, the sperm count of the surgical group showed no difference when compared to results obtained with samples from untreated boys (1). The authors concluded that early prolonged hormonal therapy is advisable in all patients with cryptorchidism, independently of surgical testicular descent into the scrotum. In their study, hormonal therapy is more effective than orchidopexy as far as obtaining adequate sperm quality in adult life is concerned (1).

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Testis tissue cryopreservation may be considered for boys with cryptorchidism

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Abstract

Cryptorchidism affects ~2–5% of full-term male infants and remains a leading cause of impaired spermatogenesis in adulthood despite contemporary surgical timing. Quantitative histology shows that a subset of cryptorchid testes have markedly reduced germ cells per tubular cross-section (G/T) in early childhood and, in the most severe cases, Sertoli-cell-only (SCO) histology—changes that predict poor fertility regardless of orchiopexy or adjunctive hormonal therapy. Preservation of future reproductive potential for prepubertal boys who cannot produce sperm therefore demands strategies that bank the spermatogonial stem cell (SSC) compartment. Since 2002, hospital programs—first pioneered in Brussels—have offered testicular tissue cryopreservation (TTC) on an experimental basis to prepubertal boys at high risk of infertility, initially those facing gonadotoxic therapies and, progressively, selected boys with cryptorchidism. Parallel laboratory advances have established: (i) long-term survival of human SSCs after xenotransplantation to murine hosts; (ii) in vitro propagation of human spermatogonia; (iii) viability and endocrine functionality of cryopreserved prepubertal human testicular tissue; and (iv) full translational feasibility in a non-human primate model culminating in a live-born rhesus macaque from sperm derived after autologous grafting of cryopreserved prepubertal testis tissue.

Over 25 years, the Copenhagen program and collaborators have contributed key clinical and laboratory milestones: defining age-sensitive germ cell depletion, demonstrating FSH-responsive ex vivo cultures, developing human TTC protocols for boys with cryptorchidism, and characterizing biomarker correlations (notably inhibin B) with G/T to guide selection. Recent prospective series from Copenhagen (2014–2022) show TTC can be integrated around orchiopexy with high parental acceptance and robust tissue quality, although clinical fertility restoration in humans remains unproven and ethically complex.

In this paper the author argues that TTC may reasonably be considered for a narrowly defined subset of boys with cryptorchidism—specifically those with bilateral disease and severely reduced G/T (≈≤0.2–0.3) or concordant biomarker profiles (very low inhibin B), after thorough counseling that emphasizes experimental status, uncertain timelines, and alternatives. The

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author synthesizes historical context, mechanistic rationale, translational evidence, clinical selection frameworks, ethics, and a pragmatic pathway to implementation within pediatric andrology services. The conclusion balances prudence with progress: offer TTC selectively today while powering registries and translational pipelines that make tomorrow's restoration options safe and real.

Key words Cryptorchidism, testis, cryopreservation

Résumé

La cryptorchidie touche ~2–5 % des garçons nés à terme et demeure une cause majeure d'altération de la spermatogenèse à l'âge adulte malgré l'optimisation du calendrier opératoire. L'histologie quantitative montre qu'un sous-groupe de testicules cryptorchides présente, dès la petite enfance, une réduction marquée du nombre de cellules germinales par coupe tubulaire (G/T) et, dans les cas les plus sévères, un aspect Sertoli-cell-only (SCO), prédictifs d'une fertilité médiocre indépendamment de l'orchidopexie ou de toute hormonothérapie adjuvante. La préservation du potentiel reproducteur chez les garçons prépubères, incapables de produire du sperme, requiert donc des stratégies visant à conserver le compartiment des cellules souches spermatogoniales (SSCs).

Depuis 2002, des programmes hospitaliers—initiés à Bruxelles—proposent la cryoconservation de tissu testiculaire (TTC) à des garçons prépubères à haut risque d'infertilité, initialement avant traitements gonadotoxiques puis, progressivement, chez certains garçons cryptorchides. Parallèlement, des avancées précliniques ont démontré : (i) la survie à long terme de SSCs humaines après xénogreffe murine ; (ii) la propagation in vitro de spermatogonies humaines ; (iii) la viabilité et la fonctionnalité endocrine du tissu testiculaire prépubère cryoconservé ; et (iv) la faisabilité translationnelle complète dans un modèle de primate non humain, avec naissance d'un macaque à partir de sperme dérivé d'un greffon autologue.

Depuis 25 ans, le programme de Copenhague et ses partenaires ont apporté des contributions essentielles : définition de l'appauvrissement germinal selon l'âge, démonstration de cultures ex vivo FSH-sensibles, développement de protocoles TTC pour la cryptorchidie, et identification d'associations biomarqueurs–histologie (notamment inhibine B ↔ G/T) pour guider la sélection. Les séries prospectives récentes (2014–2022) montrent que le TTC peut être intégré autour de l'orchidopexie, avec une forte acceptabilité parentale et une qualité tissulaire satisfaisante, bien qu'aucune restauration de fertilité humaine ne soit encore démontrée.

L'auteur soutient que le TTC peut être envisagé chez un sous-ensemble restreint de garçons cryptorchides—ceux présentant une atteinte bilatérale et un G/T sévèrement réduit (≈≤0,2–0,3) ou des biomarqueurs concordants (inhibine B très basse)—après un conseil éclairé insistant sur le caractère expérimental, les incertitudes temporelles et les alternatives. Cette contribution intègre le contexte historique, la justification mécanistique, les données

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translationnelles, les critères de sélection, les considérations éthiques et un cadre pragmatique d'implantation en andrologie pédiatrique. La conclusion conjugue prudence et progrès : offrir un TTC sélectif aujourd'hui tout en alimentant les registres et pipelines translationnels nécessaires pour rendre les options futures sûres et effectives.

Mots-clés: Cryptorchidie, testicule, cryoconservation

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Introduction

Cryptorchidism and the fertility problem

Cryptorchidism—testicular maldescent—disrupts the temperature-sensitive milieu needed for neonatal gonocyte transformation and SSC establishment. Quantitative histology from classic and contemporary cohorts demonstrates that undescended testes often have reduced germ cells per tubular cross-section, with some progressing to SCO tubules if correction is delayed beyond infancy. These lesions are strongly associated with subfertility in adulthood. Early work from Copenhagen (and collaborators) quantified these deficits across thousands of boys and highlighted a steep drop in G/T after the first year of life, strengthening international guidance for orchiopexy within 6–12 months. [1].

Despite earlier surgery, 20–25% of boys with nonsyndromic cryptorchidism are still at risk of compromised fertility potential on histological and hormonal grounds. Bilateral disease and profoundly low G/T carry the greatest risk. These observations compel us to consider fertility preservation strategies before irreversible depletion of the SSC niche. [2,3].

Why testicular tissue cryopreservation?

Prepubertal boys cannot bank semen. For adolescents who can, semen cryopreservation remains first-line. But for those before spermarche, the only way to safeguard genetic fatherhood is to bank tissue that contains SSCs—either as intact tissue fragments or isolated cell suspensions—until technologies mature to reinstate spermatogenesis. This logic mirrors the path taken by ovarian tissue cryopreservation in girls, which evolved from experimental to accepted care in many jurisdictions. The male analogue is TTC, still experimental but rapidly systematized across leading centers since 2002. [4,5].

Historical development of TTC and the cryptorchidism interface

Brussels 2002: the first prepubertal male TTC program

The Universitair Ziekenhuis (UZ) Brussel launched the world's first clinical program to bank testicular tissue for prepubertal boys at risk of infertility in 2002, initially targeting pediatric oncology and bone marrow transplantation populations. Over two decades the Brussels group standardized slow-freezing protocols, built governance and ethics frameworks, and tracked long-term safety of biopsy and pubertal development. The program has now enabled the first approved re-implantations in adult survivors, reflecting a maturation of the translational pipeline. [6,7].

From rodents to men: building the platform

Laboratory tracks progressed in parallel across species: murine organotypic cultures established cryo-methods; xenografting of human testis tissue/cells into immunodeficient mice demonstrated long-term survival and proliferation of human spermatogonia (though not full meiosis); and adult human testis studies clarified propagation conditions for

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spermatogonia. These steps forged feasibility, safety, and readouts needed for clinical protocols. [8,9].

Copenhagen contributions: culture, cryo, and selection

Well before the widespread adoption of TTC, the Copenhagen team-built ex vivo culture systems for cryptorchid testis biopsies, showing that FSH maintained tubule structure and transiently increased germ cell numbers, while LH did not confer the same benefit—suggesting a Sertoli-centric nursing effect pertinent to early human SSC biology. Subsequent work demonstrated that intact testicular tissue from young boys with cryptorchidism tolerates cryopreservation with surviving spermatogonia and sustained testis-specific hormone production in vitro-evidence that underpins the practical decision to bank tissue at the time of orchiopexy. [10,11].

Mechanistic rationale: what are we trying to preserve?

The perinatal testis undergoes gonocyte-to-spermatogonia transformation, colonizing the basement membrane as AD (type A dark) spermatogonia, a proposed SSC surrogate in early childhood. In cryptorchidism, heat stress, endocrine milieu, and local paracrine disruption impede this transformation, reducing the SSC pool and the G/T metric. Multiple studies, including Thorup and colleagues, associate low counts of PLAP-positive gonocytes and AD spermatogonia with later infertility risk. If the SSC pool is small by 6–12 months, even perfect surgical repositioning cannot fully restore it. TTC aims to bank the residual SSCs before attrition completes.[12].

Translational evidence: how close are we?

Human xenotransplantation

A critical milestone came in 2002, when adult human spermatogonial stem cells transplanted into nude mouse testes survived at least 6 months and proliferated in the first month. Colonization rates were high (>70% of recipient testes), though meiosis did not occur in the murine environment. This confirmed that human SSCs can survive cryoprocessing and transplantation and offered an assay for potency. The Copenhagen group later confirmed the technique using cryopreserved testicular tissue from prepubertal boys. [8,13].

In vitro propagation of human spermatogonia

Techniques to isolate and expand human spermatogonia have advanced steadily—from early clusters characterized by germ-cell markers to xeno-free systems and bioengineered scaffolds. Collectively, they suggest that banked human SSCs can be amplified to clinically meaningful numbers, although genomic integrity, epigenetics, and differentiation competence remain under scrutiny. [14-16].

Non-human primate proof-of-principle: "Grady"

In 2019, a Science paper reported full translational proof in rhesus macaques: prepubertal testis tissue was cryopreserved, later autografted under scrotal/back skin at puberty, matured

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for ~1 year, and then used for TESE-ICSI to achieve a live birth—the female infant Grady. This closes the loop from cryostorage to offspring in a primate whose testicular physiology approximates humans, transforming TTC from concept to demonstrated reproductive potential (albeit not yet in humans).[17].

Clinical-grade cryopreservation

Slow-freezing protocols have been optimized for clinical-grade (GMP-adjacent) conditions suitable for pediatric programs. Controlled slow-freezing with DMSO-based cryoprotectants maintains morphology, SSC marker expression, and post-thaw viability. Recent reports emphasize xeno-free media for downstream clinical translation.[18].

The Copenhagen TTC experience in cryptorchidism (2014–2022)

Program overview

From 2014 to 2022, Rigshospitalet (Copenhagen) implemented TTC around orchiopexy for 56 boys with cryptorchidism—predominantly bilateral cases—explicitly to preserve reproductive potential in those deemed at highest risk by histology and hormonal profile. Median age at orchiopexy was ~1.3 years. Germ cells were detected in ~98–100% of biopsies, with a median G/T ≈0.39 (range 0–2.88), indicating that even high-risk cryptorchid testes typically retain some SSCs to bank. [19].

Parent acceptance and counseling

Parental acceptance rates for TTC in Danish cohorts have been very high, reflecting the perceived value of "banking a chance" despite the technique's experimental status. In a 2020 study focused on cryptorchid boys, over 90% of families offered TTC consented. This aligns with similar acceptance levels in broader pediatric cohorts internationally. The counseling model is therefore crucial: obtain informed consent that covers experimental status, storage logistics, costs, and re-implantation uncertainties. [20,21].

Selecting candidates: histology and biomarkers

The Copenhagen program has long integrated histology (G/T, AD spermatogonia, SCO) and hormones to stratify risk. Inhibin B correlates positively with G/T and Sertoli-cell number; in bilateral cryptorchidism, very low inhibin B levels have a high predictive value for globally impaired G/T. These data support a biomarker-assisted selection model when biopsy is not favored or must be minimized. [22].

What the bench taught the bedside: revisiting our early experiments

The Copenhagen work with long-term organ culture of cryptorchid biopsies demonstrated that FSH (but not necessarily LH) can rescue or maintain germ cell numbers over 1–3 weeks, preserving tubule structure. This is consistent with the concept that Sertoli cells orchestrate the microenvironment for SSC survival. Although ex vivo rescue is not a clinical therapy today,

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such data de-risk culture steps before cryopreservation and in future ex vivo amplification workflows. [10].

We subsequently showed that cryopreserved prepubertal testis tissue from boys with cryptorchidism preserves SSC viability and testis-specific steroidogenic function after thaw, again validating the biological plausibility of TTC in this population. [11].

Who, when, and how: a clinical decision framework

Indications for considering TTC in cryptorchid boys today

Drawing on the translational and clinical evidence above, TTC should be considered in the following scenarios:

1. Bilateral cryptorchidism with severely reduced G/T (e.g., ≤0.2–0.3 germ cells per tubule cross-section) and/or SCO patterns on biopsy taken at orchiopexy or in a second stage procedure. Evidence suggests this group faces very high infertility risk irrespective of surgery or hormonal pretreatment. [23,24].
2. Bilateral cryptorchidism with very low serum inhibin B (age-adjusted) — especially when histology corroborates or when biopsy is constrained — given the strong correlation of inhibin B with G/T and Sertoli cell number. [22,25].
3. Parents explicitly requesting fertility preservation after informed counseling, understanding that TTC is experimental, with no current human live births from banked prepubertal human tissue (as of September 2025), but that non-human primate success exists. [6,17].

Timing relative to orchiopexy

Combine TTC with orchiopexy under the same anesthetic to minimize burden: take a small wedge biopsy (typically a few 1–3 mm fragments) from the most affected testis (or from each in bilateral cases, per protocol) when a diagnostic biopsy is clinically indicated or when histology/biomarker risk is high. This mirrors established oncology TTC workflows and leverages operating room sterility and logistics. [26].

What to bank: intact tissue vs. cell suspensions

Most pediatric centers bank intact tissue fragments using controlled slow-freezing. Some also process cell suspensions enriched for spermatogonia. Both approaches have preclinical justification; intact tissue keeps niche architecture for future autografting, while suspensions may support intratubular transplantation or in vitro spermatogenesis once clinically validated. Programs should specify standard of procedures and chain-of-custody. [27].

Cryopreservation protocol considerations

Clinical protocols typically employ DMSO-based slow-freezing with stepwise cooling, validated by post-thaw viability, morphology, and marker assays. Emerging xeno-free media address regulatory concerns. Documentation must include fragment counts, dimensions, cryoprotectant exposure, and storage location, enabling future traceability for re-implantation or lab expansion. [18].

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Restoration pathways: what could these banked tissues enable?

Autologous tissue grafting

The rhesus macaque model indicates that small tissue grafts placed in vascularized subcutaneous sites can mature through puberty to produce functional sperm. Translating this to boys demands proof of safety (no reseeding of malignancy in cancer survivors; less applicable to cryptorchidism) and efficacy (endocrine/meiotic competence). In cryptorchidism—an otherwise non-malignant indication—oncologic contamination is not a concern, potentially simplifying translation once human trials commence. [17,28].

Intratubular transplantation of SSCs

Microinjection of isolated SSCs into the rete testis or efferent ducts of a depleted testis (busulfan-treated in animal models) results in engraftment and colonization. Pediatric teams have refined fluorescence labeling and tracking in xenograft models. The future clinical use case for cryptorchid survivors could involve autologous SSC transplantation back into the now-descended testis in adulthood. [13,29].

In vitro spermatogenesis and organoids

3D organotypic cultures of thawed pediatric human tissue maintain architecture for weeks to months and can progress germ cells to spermatocyte stages in some reports. While claims of haploid cells exist, pathologic stringency remains essential; consensus is that complete human in vitro spermatogenesis has not yet been robustly demonstrated. Still, advances in scaffold design, growth factor cocktails, and xeno-free media are accelerating.[16,30].

Ethical, legal, and psychosocial dimensions

Experimental status and consent

TTC for cryptorchidism is experimental. Families must receive transparent counseling: there is no human live birth from prepubertal human tissue to date, although the primate success suggests feasibility. Consent should cover unknowns, alternative strategies (e.g., donor sperm, adoption), biopsy risks (low but non-zero), storage obligations, and future decision points. High parental acceptance rates underscore demand but must not substitute for balanced information. [6].

Equity and access

Programs must guard against inequity. TTC should be embedded in publicly accountable pathways with clear selection criteria to avoid offering an experimental procedure only to the well-resourced. International collaborations (e.g., registries, shared standard of procedures) can harmonize standards and minimize therapeutic misconception. [26].

Data stewardship

TTC creates decades-long custodianship of gametogenic tissue. Governance should codify ownership, consent at majority, disposition options, cross-border storage, and privacy. These are not abstract: Brussels' 2002 cohort is now reaching reproductive age, and initial re-

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implantations have received ethical approval—a harbinger of what cryptorchid cohorts may face in 10–20 years. [31].

Practical workflow for pediatric andrology services

Pre-operative risk stratification: Bilateral cryptorchidism, delayed surgery (>1 year), abnormal testicular volume, very low inhibin B**.** Offer shared decision-making about TTC if risk is high. [22].

Intra-operative tissue acquisition: During orchiopexy, harvest 1–3 small fragments (~1–3 mm) with atraumatic technique from the most at-risk testis. Send a diagnostic piece for histology; allocate remaining to cryo. [26].

Cryopreservation: Controlled slow-freezing with validated standard of procedures; store in liquid nitrogen; log metadata for future traceability. Consider xeno-free adaptations [18].

Documentation and follow-up: Provide families with a TTC passport (what was stored, where, for how long), and enroll in longitudinal registries**.**

Adolescent/adult transition: Reassess fertility; discuss restoration options (to be determined by future clinical trials).

Addressing concerns and counterarguments

“No human live births yet—why offer TTC?”

True—but prepubertal ovarian tissue was once in the same position and is now standard in many settings. The rhesus primate proof-of-principle shows that tissue banking can lead to live offspring in a closely related species. In cryptorchidism, tissue is benign, simplifying later fertility restoration techniques compared to oncology. Offering TTC selectively preserves options for a group otherwise facing irreversible infertility. [17,23].

“Biopsy harms the small testis.”

Complication rates of small wedge biopsies are low in experienced hands, particularly when combined with planned surgery. Longitudinal studies (e.g., Brussels) track pubertal development without overt harm, though vigilance is warranted. [32].

“Markers are imperfect.”

Yes; G/T and inhibin B are not infallible. But convergent histology + hormonal profiles highlight those at highest risk**—**precisely where the risk-benefit calculus favors offering TTC with robust consent. [3].

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"Costs and storage commitments are significant."
Correct. Programs should ensure transparent funding, equitable access, and contingency plans for storage. Research funding should shoulder much of the burden while TTC remains experimental. [4].

Future directions: from banked tissue to babies

1. **Clinical trials of autografting in benign indications.** Cryptorchidism lacks oncologic contamination risk; carefully designed first-in-human autografting under ethics oversight is a logical next step as methods mature. Recent approvals and first re-implantations in oncology survivors demonstrate regulatory feasibility. [7,31].
2. **Standardized readouts.** Beyond histology, develop functional assays (e.g., molecular maturation indices, single-cell omics) to predict graft potential and screen for genetic/epigenetic stability post-culture. [33].
3. **Xeno-free propagation pipelines.** Scale cleanroom-compatible SSC expansion with defined media; build bioreactors and quality release criteria (karyotype, epigenetics, off-target differentiation). [18].
4. **Biomarker algorithms.** Integrate inhibin B, FSH, testis volume, and G/T into a risk calculator to standardize TTC offers. [3,34].
5. **Global registries.** Share safety, acceptance, tissue metrics, and outcomes across centers to accelerate learning and ensure equity. [26].

Integrating the symposium transcript insights

The narrative from Vassalli Hall captures the arc of this field:

> 2002 Brussels program inaugurates TTC for prepubertal boys facing gonadotoxic therapies, inspired by ovarian tissue success. [6].
>
> Early Copenhagen culture experiments show FSH supports germ cells in cryptorchid biopsies; subsequent cryopreservation studies demonstrate post-thaw viability and hormone production in vitro. [10,11].
>
> Adult human work in Philadelphia demonstrates long-term survival of human SSCs in nude mouse testes, establishing the key potency readout. [8].
>
> Propagation of human spermatogonia in vitro evolves to cluster-based systems and, later, xeno-free conditions in Copenhagen's post-doctoral projects. [14,15].
>
> Winston-Salem and other centers implement clinical TTC around 2014–2016, reporting high parental acceptance. Copenhagen's cryptorchid cohort mirrors this pattern. [20,21].

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The 2019 rhesus macaque breakthrough—Grady—establishes end-to-end feasibility from cryopreserved prepubertal tissue to offspring. [17].

Biomarker work consolidates inhibin B as a practical correlate of G/T, offering a less invasive path to selection. [22].

These points reinforce the thesis: selective TTC is a defensible, ethically sound offer to certain cryptorchid boys today, with a credible translational horizon.

Conclusion

Cryptorchidism continues to confer substantial infertility risk for a meaningful minority of affected boys, predominantly those with bilateral disease and severe early germ cell depletion. While orchiopexy by 6–12 months mitigates risk, it cannot restore an SSC reservoir that was never properly established. Over the last 25 years, a translational pipeline has cohered: human SSC survival in xenografts, in vitro propagation, validated cryopreservation, and a non-human primate live birth from cryopreserved prepubertal testis tissue. Clinical programs—first in Brussels and subsequently worldwide—have embedded TTC into pediatric care for high-risk groups, with Copenhagen extending this rationale to selected cryptorchid boys based on histology and biomarkers.

Given this evidence, it is reasonable today to offer TTC to a narrowly defined subgroup of boys with cryptorchidism—bilateral, severely low G/T (≈≤0.2–0.3) or very low inhibin B—provided families consent with full understanding that (i) TTC is experimental, (ii) no human births from such tissue have yet been reported, and (iii) restoration is most plausibly via autografting or SSC transplantation as clinical trials emerge. This approach preserves future options without overpromising, advances equitable access via structured protocols, and aligns with a 20-year horizon in which at least one restoration pathway is likely to become clinically usable. In other words, banking potential now is a rational step toward realizing fertility later for the most vulnerable cryptorchid boys.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests. This article is AI generated based on a recording of the lecture with accompanying slides presented by the author in the Vassalli Hall, International Conference Center, Valletta, Malta, 26 September 2025. The manuscript is reviewed and corrected by the author.

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Buserelin (GNRHa)-treatment; three-arm placebo-controlled study

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Abstract

Background. Cryptorchidism (undescended testis, UDT) affects ~2–3% of term male infants and remains the most common genital anomaly of childhood. Beyond malposition, the central clinical problem is impaired germ cell maturation during the **mini-puberty** window (postnatal surge of GnRH–LH/FSH–testosterone), which in a subset of boys fails to occur, leading to loss of **adult dark (Ad) spermatogonia**, the stem-cell–like population that predicts future fertility. Surgical repositioning (orchiopexy) addresses location, but not necessarily the endocrine milieu. In the late 1980s–early 1990s, we therefore tested whether a brief course of intranasal **buserelin** (a GnRH agonist) could (a) induce descent and (b) **rescue germ-cell maturation** in cryptorchid boys.

Methods. In a randomized, double-blind, placebo-controlled, **three-arm** trial at the Federal University Children's Hospital (UFRJ, Rio de Janeiro) with blinded histology processed in Basel, boys with true cryptorchidism were assigned to: (i) placebo; (ii) intranasal buserelin **20 µg once daily for 28 days**; or (iii) primary surgery (orchiopexy). Subsequently, undescended testes underwent orchiopexy with **bilateral testicular biopsies** in all participants to quantify germ cells and maturation indices. Design, dosing, blinding, and histology endpoints are detailed in the primary publications and reiterated in the symposium program and transcript.

Results. In the published trial **59 boys** were randomized (22 buserelin, 18 surgery, 19 placebo). Buserelin significantly increased scrotal descent compared with placebo ($p < 0.01$). Boys treated with buserelin had **the highest number and maturation index of germ cells**; epididymal development and closure of the processus vaginalis were also more favorable in the buserelin group ($p < 0.003$ and $p < 0.05$, respectively). The surgical arm appropriately repositioned testes but, as expected, did not itself reproduce the endocrine environment of mini-puberty. Subsequent follow-up and translational work showed that presence of **Ad spermatogonia** after endocrine therapy predicts better fertility potential; conversely, boys lacking Ad spermatogonia (impaired mini-puberty) face persistently reduced fertility prospects despite anatomically successful surgery.

Conclusions. A short, low-dose buserelin course can, in carefully selected boys, (i) induce descent when the testis is near the scrotum and (ii) support **germ-cell maturation**, a

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biologically meaningful endpoint that surgery alone cannot provide. Modern guidelines still recommend **early orchiopexy (by 6–18 months)** and generally **discourage routine hormonal therapy for descent** because population-level response rates are modest and heterogeneous; however, converging histologic and molecular data indicate that **adjuvant GnRH-agonist treatment** may be valuable for the **subset** with endocrine-mini-puberty failure and poor Ad-spermatogonia counts. This talk revisits the design, outcomes, and lessons of the Rio–Basel RCT, addresses controversies, and frames a pragmatic, risk-stratified approach that integrates histology with contemporary care pathways.

Key Words Cryptorchidism, treatment, Buserelin, three arm placebo-controlled study, histology, Ad spermatogonia

Résumé

Contexte. La cryptorchidie, présente chez 2–3 % des nouveau-nés masculins, compromet la maturation germinale lorsque la mini-puberté est insuffisante, entraînant la perte des spermatogonies Ad, marqueurs clés du potentiel fertile. L'orchidopexie corrige la localisation testiculaire mais ne restaure pas l'environnement endocrinien. Nous avons évalué l'effet d'un agoniste de la GnRH sur la descente testiculaire et la maturation germinale.

Méthodes. Dans un essai randomisé, en double insu et contrôlé par placebo, trois stratégies ont été comparées : placebo, busséréline intranasale (20 µg/j, 28 jours) et orchidopexie primaire. Des biopsies testiculaires bilatérales ont été réalisées pour quantifier les cellules germinales et les marqueurs de maturation.

Résultats. Parmi 59 participants, la busséréline a significativement accru la descente scrotale ($p < 0,01$) et amélioré les indices de maturation germinale ainsi que le développement épididymaire ($p < 0,003$). La chirurgie seule n'a pas reproduit le contexte endocrinien de la mini-puberté. Le suivi a confirmé que la présence de spermatogonies Ad après traitement endocrinien prédit un meilleur potentiel de fertilité.

Conclusions. Un bref traitement par busséréline peut, chez des garçons sélectionnés, favoriser la descente testiculaire et restaurer la maturation germinale, un bénéfice non obtenu par la chirurgie seule. Bien que les recommandations actuelles privilégient l'orchidopexie précoce, les données récentes suggèrent qu'une thérapie adjuvante par agoniste de la GnRH pourrait bénéficier au sous-groupe présentant un échec endocrinien de la mini-puberté.

Mots-clés : Cryptorchidie, traitement, Busséréline, étude à trois bras contrôlée par placebo, histologie, spermatogonies Ad

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Introduction: the clinical problem the trial set out to solve

Cryptorchidism is not merely a problem of anatomy. Even when a surgeon places the testis into the scrotum, **the future of spermatogenesis** depends on whether the boy experienced an adequate **mini-puberty**—the transient activation of the hypothalamic–pituitary–gonadal axis during the first months of life—because that hormonal surge drives **Ad spermatogonia** formation and Sertoli-cell maturation [1-3,5]. Absent or blunted mini-puberty is strongly associated with low Ad-spermatogonia counts on biopsy and **poor adult fertility**, even after timely, technically successful orchiopexy [1-3,5,8]

In the late 1980s, two clinical intuitions converged:

1. **If** a GnRH agonist could transiently reproduce (or "replace") the endocrine features of mini-puberty in toddlers and preschoolers with UDT, it might stimulate **germ-cell maturation** and **improve the fertility index**; and
2. Some testes positioned near the scrotum might achieve **descent** under such hormonal stimulation, potentially simplifying surgery and optimizing epididymal maturation and processus vaginalis closure [1,2].

These insights motivated our **randomized, double-blind, placebo-controlled three-arm** study in Rio de Janeiro with **blinded histology in Basel**, reported in *The Journal of Urology* (1992) and *European Journal of Pediatrics* (1993) [1,2]. The present paper synthesizes those results, integrates later evidence on Ad spermatogonia biology, and situates buserelin therapy within today's guideline landscape [3-6,9-13].

Methods in brief (Rio–Basel RCT)

Trial design and setting

Prospective, randomized, double-blind, placebo-controlled trial with three arms: **buserelin**, **placebo**, and **primary surgery**. All clinical care and randomization occurred at the Federal University Children's Hospital (UFRJ, Rio de Janeiro); **histology was processed blinded** in Basel to prevent observer bias, with codes opened **after** histologic assessments were complete (1,2).

Participants

Boys with **true** cryptorchidism were enrolled following exclusion of retractile testes after careful examination. In the published report, **59 boys** were randomized: 22 to buserelin, 18 to primary surgery, 19 to placebo (1,2). (Contemporaneous remarks recall a slightly different screened or eligible tally; differences likely reflect pre-randomization exclusions versus randomized participants, consistent with the final *J Urol* numbers (1,2).

Interventions

- **Buserelin arm: 20 µg** intranasal **once daily for 28 days** (1,2).
- **Placebo arm:** matched intranasal spray for 28 days.
- **Surgery arm:** orchiopexy without preceding hormonal therapy.
  After medical therapy, testes that remained undescended underwent orchiopexy.

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Bilateral biopsies were obtained in **all** participants (including responders) to quantify germ-cell counts and maturation indices; epididymal status and processus vaginalis were assessed (1,2).

Outcomes

Primary biological outcome: germ-cell maturation, particularly the **number of germ cells per tubular cross-section** and maturation index; the presence of **Ad spermatogonia** was emphasized in later follow-ups (1–6,8).

Clinical outcomes: testicular position/descent (pre-scrotal/scrotal), epididymal morphology, and processus vaginalis closure (1,2).

Blinding and analysis

Randomization and drug assignment were blinded to surgeons and pathologists; **codes were opened only after histology** (1,2). Group comparability at baseline (age, bone age, anthropometrics, hormones, penile size, contralateral testis volume) was confirmed (1,2).

Results

Descent and anatomic maturation

Descent: Buserelin significantly increased scrotal descent vs placebo ($p < 0.01$). Most responses occurred in **pre-scrotal** or low inguinal positions; higher intra-inguinal/abdominal testes showed limited response—an observation echoed in the talk Q&A (1,2).

Epididymis: Normal epididymis was more frequent among boys with successful descent ($p < 0.003$) (1).

Processus vaginalis: Closure occurred more often after buserelin than after primary surgery alone ($p < 0.05$) (2). These anatomic maturational effects are biologically consistent with androgen-dependent epididymal development and gubernacular/processus changes (2,4).

Histology: germ-cell number and maturation

Buserelin-treated boys exhibited **the highest germ-cell counts** and **maturation indices** among the three arms (1). In the trial, the **median/mean germ-cell counts** per tubule improved meaningfully in the buserelin group compared to placebo or immediate surgery; later commentary from the investigator notes a mean around **0.84 germ cells per tubule** after 28 days of buserelin, significantly greater than comparator arms, in keeping with the published statement that buserelin increased the number and maturation of germ cells (1). Crucially, **histology was blinded**, preventing expectation bias (1,2).

Ad spermatogonia and fertility prognosis

Subsequent work from Basel and collaborators defined **Ad spermatogonia** as a **key histologic marker** of completed mini-puberty and future fertility potential (3–6,8). In follow-ups, boys **with sufficient Leydig-cell capacity** (i.e., adequate testosterone response) achieved **normal Ad-spermatogonia counts** after hormonal treatment; those with **suboptimal Leydig function** did **not**, even if surgery succeeded anatomically (5). These data sharpen our interpretation of the RCT: the **germ-cell benefit** of buserelin likely concentrates

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in the subgroup with **mini-puberty deficiency yet sufficient capacity to respond** to GnRH-agonist stimulation (3–6,8,14,15).

How the Rio–Basel trial fits with the wider evidence (then and now)

4.1 Historical context

Earlier hormonal trials using **hCG** or **GnRH** showed variable descent rates with **frequent re-ascent**; meta-analyses in the 1990s–2010s placed overall sustained descent from hormonal therapy near **15–20%**, far below surgery (~95%) (11,12). A 1992 multicenter study even suggested hCG outperformed GnRH for **descent**, though both improved as age decreased (10,11). However, almost none of those trials were designed or powered to detect **histologic maturation**—the core biological endpoint our trial prioritized (1,2,10–12).

Modern guideline positions

Guidelines from major societies (AUA, EAU/ESPU, and international comparisons) **recommend early orchiopexy** (ideally by **6–12 months**, and no later than **18 months**) and **do not recommend routine hormonal therapy to induce descent**, citing modest and inconsistent response and potential germ-cell harm in nonresponders (9). That said, contemporary reviews acknowledge a biologic rationale for **adjuvant GnRH-agonist** in **selected** boys to improve **fertility index/Ad-spermatogonia**, particularly when used **pre- or peri-operatively** and evaluated within a structured protocol (6,11,16). This targeted, risk-stratified position is compatible with our trial's central message: **surgery is the cornerstone**, but **endocrine rescue** can matter for **germ cells** (1,2,6,9–13).

Mechanism: mini-puberty rescue and cellular programs

Mini-puberty physiology. After birth, pulsatile GnRH stimulates LH/FSH, transiently increasing **testosterone** and **inhibin B**, driving Sertoli- and germ-cell maturation and **Ad-spermatogonia** formation (3–5). In some cryptorchid boys, this surge is **insufficient**, leaving a lasting "imprint" of low Ad-spermatogonia and impaired fertility potential (3,5).

GnRH-agonist action. Short-course **buserelin** can, after an initial flare, enhance gonadotropin/testosterone exposure sufficiently to **promote germ-cell maturation** and **epididymal/processus development** (1,2,4). Molecular studies report recovery of Ad-spermatogonia and coordinated changes in **PRDM** family gene expression and Y-chromosome spermatogenesis genes after GnRH-agonist therapy, linking endocrine rescue to spermatogonial stem-cell programs (7). These data support a **causal chain**: GnRH-agonist → mini-puberty–like milieu → Ad-spermatogonia rescue → improved fertility index (3,4,6,7,16).

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Practical lessons from the three-arm RCT

Lesson 1 — Orchiopexy remains the cornerstone. Timely surgery relocates the testis, reduces thermal stress, and lowers malignancy risk; it is the **standard of care** (9). Hormonal therapy does **not** replace surgery in bilateral high intra-inguinal or abdominal testes, and it should never delay recommended surgical timing.

Lesson 2 — Histology matters. Biopsies (intraoperative or protocolized) provide **prognostic insight** about **Ad-spermatogonia**. In our RCT, blinded histology demonstrated meaningful germ-cell benefits from buserelin beyond mere positional change (1,2). Where expertise allows and risks are acceptable, **biopsy-informed counseling** is appropriate, especially in higher-risk phenotypes (bilateral UDT, high position, small testis) (3–6,8–11).

Lesson 3 — Patient selection. The **best endocrine responses** occur in **low-position** testes (pre-scrotal/low inguinal) and in boys with **residual Leydig-cell capacity** (1,2,5). These are the children most likely to benefit from a **brief, low-dose GnRH-agonist** protocol aimed at **maturation**, not just descent.

Lesson 4 — Protocol discipline and blinding. Randomization, strict blinding, centralized histology, and well-defined endpoints made our study unusually robust for its era. The **28-day 20 µg/day intranasal** regimen was deliberately conservative—enough to test the biological hypothesis, short enough to minimize exposure (1,2).

Lesson 5 — Integrating modern consensus. Today, routine hormonal therapy to induce descent is **not** advised; nevertheless, **adjuvant endocrine therapy** can be **considered** in a **defined subset** to address **mini-puberty failure** and improve the **fertility index**, always within shared decision-making and without deferring timely surgery (6,9–13).

A risk-stratified care pathway (synthesis)

1. **Early identification** (by 6 months corrected age). Avoid routine imaging; clinical exam suffices (9,13).
2. **Refer for orchiopexy** by 6–12 months (no later than 18 months) (9,13).
3. **Consider biopsy-guided counseling** at orchiopexy in bilateral UDT, high position, small/discordant testis, or syndromic contexts (3–6,8–11,13).
4. **Targeted endocrine adjuvant** (short-course GnRH-agonist such as buserelin) **may be considered** to address **germ-cell maturation** in boys with histologic evidence of poor Ad-spermatogonia and suspected mini-puberty failure—ideally in a protocol with outcome tracking (3–6,7,14,16).
5. **Follow-up** includes testicular position/size, puberty progression, and, when appropriate, adolescent fertility counseling.

This pathway honors **guideline standards** while preserving the **biological insight** of the Rio–Basel trial: **some** boys need **more than relocation**—they need **mini-puberty replacement** to secure a better spermatogonial future (1–6).

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Limitations and controversies

- **Heterogeneity of cryptorchidism.** UDT is a **syndrome** with diverse etiologies (anatomical, endocrine, genetic). Aggregate descent rates dilute subgroup benefits (11–13).
- **Re-ascent.** Early hormonal descent can be followed by re-ascent; hence, surgery remains necessary for many (11,12).
- **Evidence standards.** Few RCTs assessed **histology** with **central blinding**; our trial is an exception. The field needs modern trials that embed **molecular endpoints** (PRDM signaling, MSY gene expression) and **long-term semen analyses** (1,2,7,16,17).

Conclusions

The Rio–Basel **three-arm, double-blind, placebo-controlled** trial demonstrated that a **brief, low-dose buserelin** course can induce descent in low-lying testes **and**—more importantly—**enhance germ-cell maturation** measured by blinded histology (1,2). Thirty-plus years later, the biologic signal we sought to test—**mini-puberty replacement**—has found support in **histologic**, **molecular**, and **long-term** observations around **Ad spermatogonia** and fertility potential (3–8,16,18). While **orchiopexy** remains the **non-negotiable core** of management, a **selective, biopsy-informed** use of **GnRH-agonists** deserves a place in nuanced care pathways for boys at **highest fertility risk**.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Replacement of Male Mini-Puberty

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Abstract

Background. "Mini-puberty" is the transient activation of the hypothalamic–pituitary–gonadal (HPG) axis from ~2 weeks to 3–6 months in boys, driving Leydig- and Sertoli-cell activity, penile and testicular growth, and programming of future fertility. In **congenital hypogonadotropic hypogonadism (CHH)**—including Kallmann syndrome—this surge is absent, leading to **micropenis** and **bilateral cryptorchidism** despite otherwise normal anatomy. Because classical treatment paradigms address phenotype later (testosterone for penile length; surgery for testicular position), they often miss this early **physiologic window** and may not fully rescue fertility potential.

Objective. To synthesize the rationale, physiology, and clinical evidence for **replacement of male mini-puberty** using **exogenous gonadotropins (recombinant LH/FSH)** and related strategies, anchored in the author's REMAP program and subsequent literature.

Methods & Sources. Narrative review with emphasis on endocrine physiology, translational studies, and prospective case series/registries. Core primary sources include the author's **REMAP** study ("Replacement of Male Mini-Puberty"), ESPE abstracts, and the ISRCTN registry, alongside contemporary reviews and consensus statements.

Findings. In neonates/infants with CHH and absent mini-puberty, **daily subcutaneous recombinant LH/FSH** (typically **75/150 IU**; **Pergoveris®**) for **3 months** reproducibly: (i) raises LH/FSH to postnatal physiologic ranges; (ii) drives **testosterone** into pubertal levels; (iii) normalizes **inhibin B** and **AMH**; (iv) increases **penile length** from <2 cm to ≈3.5–4.0 cm median; (v) induces **testicular descent** to the scrotum or low inguinal canal with volumes ~1.5–2.5 mL; and (vi) initiates **catch-up growth**—all with acceptable short-term safety. Limited follow-up suggests sustained scrotal position in most; occasional low inguinal re-migration is manageable surgically. Mechanistically, therapy **mimics the physiologic surge**, restoring Leydig/Sertoli interplay that can never be recaptured by late adolescence-onset induction alone.

Conclusions. For **appropriately selected** boys with **documented absence of mini-puberty** and **CHH** (especially those with bilateral non-palpable testes and micropenis), **gonadotropin replacement** in early infancy provides a physiologic, noninvasive means to repair micropenis, facilitate or complete testicular descent, and likely **preserve fertility potential**. Programs should be embedded within multidisciplinary DSD/andrology networks with standardized diagnostics, dosing, monitoring, and long-term outcome registries.

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Key words Cryptorchidism, hyogonagotrope hypogonadism, pergoveris treatment

Résumé

Contexte. La « mini-puberté » correspond à l'activation transitoire de l'axe hypothalamo–hypophyso–gonadique (HPG) entre ~2 semaines et 3–6 mois chez le garçon, stimulant l'activité des cellules de Leydig et de Sertoli, la croissance pénienne et testiculaire, ainsi que la programmation de la fertilité future. Dans l'hypogonadisme hypogonadotrope congénital (CHH), incluant le syndrome de Kallmann, cette activation est absente, entraînant micropénis et cryptorchidie bilatérale malgré une anatomie par ailleurs normale. Les approches classiques, intervenant tardivement, ne restaurent pas cette fenêtre physiologique précoce.

Objectif. Présenter la physiologie, la justification et les preuves cliniques du remplacement de la mini-puberté masculine par gonadotrophines exogènes (LH/FSH recombinantes), en s'appuyant sur le programme REMAP et la littérature ultérieure.

Méthodes. Revue narrative centrée sur la physiologie endocrinienne, les données translationnelles et les séries prospectives provenant notamment de REMAP, des abstracts ESPE, du registre ISRCTN et de revues/consensus contemporains.

Résultats. Chez les nourrissons CHH dépourvus de mini-puberté, l'administration quotidienne de LH/FSH recombinantes (≈75/150 UI pendant 3 mois) : (i) restaure des taux physiologiques de LH/FSH ; (ii) élève la testostéronémie à des niveaux pubertaires ; (iii) normalise l'inhibine B et l'AMH ; (iv) augmente la longueur pénienne (<2 cm → ≈3,5–4,0 cm) ; (v) induit une descente testiculaire avec volumes de 1,5–2,5 mL ; et (vi) initie une croissance de rattrapage, avec un profil de tolérance satisfaisant. Les données limitées de suivi suggèrent une stabilité majoritaire de la position scrotale.

Conclusions. Chez les garçons soigneusement sélectionnés présentant une absence documentée de mini-puberté et un CHH (notamment en cas de micropénis et de cryptorchidie bilatérale), le remplacement gonadotrope précoce offre une stratégie physiologique, non invasive, pour corriger le micropénis, faciliter la descente testiculaire et potentiellement préserver la fertilité. Ces programmes doivent être intégrés à des réseaux DSD/andrologie avec protocoles standardisés et registres longitudinaux.

Mots-clés: Cryptorchidie, hypogonadisme hypogonadotrope, traitement par Pergoveris

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Introduction and Scope

Mini-puberty is a **time-limited, developmentally programmed** burst of GnRH-driven gonadotropin secretion in early infancy. In boys, LH rises within 1–2 weeks, peaks at 1–3 months, then wanes by 4–6 months; FSH peaks slightly later; testosterone follows LH by ~1 week, reaching pubertal levels around 1–3 months before returning to prepubertal levels by ~6 months [1-9]. This signal underwrites **Leydig-cell proliferation**, **testosterone production**, **Sertoli-cell proliferation** (despite immature androgen receptor expression), **inhibin B** production, and **AMH** dynamics; clinically, it supports **penile growth**, **testicular growth**, **epididymal/wolffian maturation**, and aspects of **neurobehavioral sexual differentiation** [6–9,11,14,16].

In **CHH**, the surge is **absent**. Affected boys present with **micropenis** and **bilateral cryptorchidism** and, left uncorrected endocrinologically, risk **persistent Sertoli-cell immaturity**, poor germ-cell endowment, and **infertility** even when orchiopexy is technically successful [6–9,14,16,17]. Traditional care—**testosterone injections** for micropenis and **two-stage orchiopexy**—addresses anatomical and short-term phenotypes but **does not replace the physiology** that mini-puberty supplies.

This paper advances a **physiology-first** approach: **replace** mini-puberty **when the body expects it**—with gonadotropins, not just androgens—so that the **Leydig/Sertoli axis** learns the proper "language" and the **testes** mature in the right hormonal context [1–3,10,12,13,15,16,18,19].

Physiology Refresher: What Mini-Puberty Does (and Why Pulses Matter)

Pulsatile GnRH. GnRH receptor signaling in pituitary gonadotropes requires **pulsatility**; continuous occupancy down-regulates receptors and suppresses gonadotropins (as exploited by depot GnRH analogs to suppress puberty) [6–9]. Peripheral cues (leptin, insulin/IGF-1, ghrelin) modulate GnRH neurons and kisspeptinergic inputs, aligning growth and energy balance with reproductive timing [6–9]. The Vassalli Hall remarks stressed the "**push-and-release**" nature of GnRH—akin to a light switch repeatedly pressed—needed to stimulate **LH/FSH** release.

Fetal and neonatal timing. Fetal LH/FSH production emerges by 9–12 weeks; **hCG** dominates early Leydig stimulation; by mid-gestation, testosterone peaks at **pubertal levels** and falls thereafter. Neonatally, the **second activation** (mini-puberty) is GnRH-dependent and **independent of gestational age at birth**; in preterms, it starts at the same **postnatal** age, often **lasts longer** and is **more intense** [6–9,16,17].

Cellular consequences. Mini-puberty promotes **Leydig-cell proliferation** and **testosterone** secretion, **Sertoli-cell proliferation** (rising **AMH/inhibin B** despite limited androgen receptor expression), **increases testicular volume**, contributes to **inguinoscrotal descent**, and correlates with **penile growth** and early **sex-typed behaviors** [6–9,10,16,17].

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Why replace it? If mini-puberty **fails** (as in CHH), boys are "born late": the testis never sees the **right conversation** between LH/FSH and Sertoli/Leydig cells. Waiting until puberty and "speaking only testosterone" cannot fully remedy what required **two voices** (LH and FSH) in infancy [6–9,10,16]. Hence **replacement** aims to restore **both** signals at the **right time**.

The Diagnostic Window You Can't Afford to Miss

When: 15 days to **3–6 months** in boys is the **unique diagnostic window** to **prove or refute** CHH via **two blood samples** (e.g., at ~2–4 weeks and ~6–10 weeks): LH, FSH, testosterone, **inhibin B**, **AMH**, ± DHT; screen adrenal function as indicated. In CHH, **LH/FSH** and **testosterone** are **undetectable/very low**; inhibin B and AMH are often low for age [6–9,16,17].

Who: Any boy with **micropenis** (stretched penile length < **2.5 cm** at term) and/or **bilateral cryptorchidism** should prompt urgent endocrine evaluation [6–9,16,20]. The transcript underscores that **even NICU preterms** share the same **postnatal timing—call endocrinology** or the window will be lost.

Why it matters for DSD: Beyond CHH, mini-puberty testing refines evaluation of **46,XY DSD**, **46,XX DSD**, and **sex chromosome aneuploidies**; it informs sex assignment and surgical timing through a **one-tube workup** aligned to imaging and karyotype [6–9,10,16].

The Clinical Problem in CHH: Micropenis + Bilateral Cryptorchidism

CHH prevalence is low, but among boys with **bilateral UDT + micropenis**, an **endocrine etiology** is common. **Kallmann syndrome** (CHH with anosmia) and **normosmic CHH** are genetically heterogeneous (dozens of genes governing GnRH neuron development/migration/function) and can show **variable** associated pituitary and neurodevelopmental features [7–9,17]. Absence of mini-puberty translates to **hypoplastic testes** (difficult to find or mobilize), **micropenis**, and a **high burden of surgery** if endocrine physiology is not restored [7–9,18].

Therapeutic Options: What Works, What's Physiologic, and When

Testosterone injections

Use-case: Micropenis only, or as adjunct before hypospadias repair. **Pros:** Simple, inexpensive; **15 mg** IM every 4–12 weeks x 2–3 doses is traditional; enlarges penile length without altering final adult size or bone age. **Cons: Does not** stimulate Sertoli cells or recreate the **biphasic** Leydig–Sertoli dialogue; **won't** descend high intra-abdominal testes; **won't** normalize inhibin B/AMH. It fixes the **look**, not the **system** [6–9,10,16].

Pulsatile GnRH

Conceptually physiologic but **impractical** in neonates; requires a pump and intact pituitary; experience is limited [6–9].

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Gonadotropin replacement (LH + FSH) — the REMAP approach

Rationale: Replace **both** pituitary outputs (**LH/FSH**) to **mimic mini-puberty** during the **exact** window it should occur.

Protocol: Daily **subcutaneous** recombinant **LH/FSH** (commonly **75/150 IU**; **Pergoveris®** pen now available with 150/300 options) for **3 months**; evening dosing aligns with half-lives [1–3,12].

Mechanism: LH → Leydig testosterone; FSH → Sertoli proliferation/inhibin B/AMH; jointly promote **testicular growth**, **penile growth**, and **descent** [1–3,6–10,12,13,15,16,18,19].

Evidence (REMAP & related):

- **Design:** Prospective single-center cohort/registry (ISRCTN13007297), 2009–2019, neonates/infants with **absent mini-puberty**, **micropenis**, **bilateral non-palpable testes** [1–3,12,13,16,19].
- **Dosing: Daily** LH 75 IU + FSH 150 IU SC x **3 months**; total doses ≈ **6,750 IU LH** and **13,500 IU FSH** [1–3,12,13,16].
- **Hormonal response:** LH rose to high-normal median ~4.5 IU/L; FSH supranormal (~80 IU/L); testosterone to ~3.3 ng/mL; **inhibin B/AMH** normalized—consistent with **physiologic mini-puberty** [1–3,12].
- **Anatomic response: All testes** descended to **scrotal** or **low inguinal** positions during therapy with volumes **~1.5–2.5 mL**; **penile length** increased from <2 cm to median **~3.8 cm** [1–3,12].
- **Durability:** Over 1–10 years, most remained scrotal; a minority re-migrated to **low inguinal** within a year and were easily **fixed** surgically [1–3,12].
- **Safety: No significant adverse events**; post-therapy scrotal ultrasounds were **normal** [1–3,12].

These outcomes reproduce the **physiologic phenotype** of mini-puberty across hormones and anatomy, with **functional** implications for future spermatogenesis that purely **androgenic** or purely **surgical** approaches cannot ensure [1–3,6–9,12].

Protocolization: A Practical "REMAP-Style" Pathway

Eligibility

- Neonate/infant (0.2–0.8 y typical in REMAP) with **micropenis** and **bilateral non-palpable testes** AND **documented absent mini-puberty**: undetectable/low **LH/FSH**, **testosterone**, low **inhibin B/AMH** on **two** samples [1–3,12].
- Imaging (US ± MRI) to confirm intra-abdominal/inguinal position; evaluate pituitary and olfactory structures when Kallmann suspected.

Dosing

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- **Pergoveris®** (recombinant **LH 75 IU** + **FSH 150 IU**) **SC daily x 3 months**; train caregivers for home injections; dose in the **evening** [1–3,12].
- **Total dose ≈ 6,750 IU LH** and **13,500 IU FSH** [1–3,12–13,16].
- Consider **pen presentations** (150/300) for easier titration; future comparative work may test **1:1 vs 2:1 FSH:LH** ratios .

Monitoring

- Monthly: **LH**, **FSH**, **testosterone**, **inhibin B**, **AMH**; anthropometrics; **stretched penile length**; **testicular volume/position** [1–3,12].
- Safety: Injection-site reactions, hematocrit, liver panel as local policy dictates; ultrasound at end of therapy [1–3,12].

Endpoints

- **Hormonal:** Attainment of **pubertal-range testosterone** for age; **normalization** of **inhibin B/AMH**.
- **Anatomic:** Descent to **scrotum/low inguinal**, **penile length** ≥ −2 SD for age; **testicular volume** ~1.5–2.5 mL [1–3,12].
- **Follow-up:** Annual monitoring of testicular position/size; if any re-migration to low inguinal, **simple orchiopexy** suffices [1–3,12].

Special Populations and Nuances

- **Preterm infants:** Same **postnatal** timing; replacement may need **longer** duration if the endogenous surge is prolonged/intense; evidence is emerging [6–9,16].
- **Syndromic CHH/complex DSD:** MRI often reveals olfactory tract/bulb hypoplasia in **Kallmann**; septo-optic dysplasia and other pituitary malformations require broader pituitary replacement and neurology input [7–9].
- **Prader–Willi syndrome:** Pilot use suggests LH/FSH can mobilize high testes to inguinal canal and amplify penile growth, facilitating simpler surgery; formal trials needed.
- **Genetics:** >20–40 implicated genes (reviews vary) with variable penetrance; genetic testing adds prognostic value but **must not delay** treatment during the **tiny window** [7–9,11].

How Strong is the Evidence?

- **Prospective case series/registry (REMAP)** with **1–10 years** follow-up; reproducible hormonal/anatomic responses; manageable surgical needs thereafter [1–3,12–19].

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- **Convergent reviews** endorse **mini-puberty replacement** as rational and promising for **CHH**; a 2024 Endocrine Reviews piece outlines algorithms for **early diagnosis** and discusses replacement options [6].
- **Case-level fertility outcomes** after **induced mini-puberty** exist, but **long-term semen analyses** following infancy LH/FSH replacement remain limited—an urgent research gap [18].
- **Safety:** Short-term safety favorable; no signal for adverse growth plate acceleration or metabolic effects in infancy; formal pharmacovigilance registries recommended [1–3,12].

Health-System Implementation

- **Trigger:** Any **bilateral UDT** and/or **micropenis** → automatic endocrine consult **within 2–3 weeks** of life.
- **Pathway integration:** Standardized orders for **mini-puberty labs**, imaging, and **expedited payer approval** for LH/FSH pens.
- **Training:** Nurse-led caregiver education for **home injections**; helpline for dosing/AE queries.
- **Data:** Enroll in a **prospective registry** (ISRCTN/clinical trials) for standardized outcomes (testis position, volumes, hormonal profiles, later pubertal progression, semen parameters).

Controversies & Counterpoints

- **"Why not just operate?"** Surgery relocates testes but **cannot** induce **Sertoli maturation** or recapitulate endocrine programming; CHH boys remain at **infertility risk** without hormonal rescue [6–9,14,16].
- **"Isn't testosterone enough?"** It **enlarges the penis** but under-serves Sertoli biology, **inhibin B**, **AMH**, and **germ-cell milieu** [6–9,14,16].
- **"Are supraphysiologic FSH levels a concern?"** Transient supranormal **FSH** reflects pharmacokinetics and is **expected**; no adverse signals reported in REMAP; still, structured monitoring is prudent [1–3,12].
- **"Evidence level?"** Randomized trials are challenging in rare neonatal CHH; multicenter pragmatic studies with harmonized outcomes are feasible and overdue [6–9,14,16].

Conclusions

Mini-puberty is **not optional**—it is **instructional**. In **CHH**, the instruction set is **missing**, and **time-aligned replacement** is the only way to convey it. The **REMAP** experience demonstrates that **daily LH/FSH** in early infancy can **repair micropenis**, **induce/assist descent**, **normalize Leydig/Sertoli biomarkers**, and **launch** a healthier developmental trajectory with minimal

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burden to families. Implementation requires **systems that move fast**, **teams that coordinate**, and **registries that learn**. The payoff is substantial: fewer operations, better anatomy, and—most importantly—a more **fertility-capable** testis for adult life.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Molecular evidence supports hormonal treatment for cryptorchidism

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Abstract

Combining histology, endocrinology, and transcriptomics, this paper presents molecular proof that mini-puberty failure—not malposition alone—causes infertility in many cryptorchid boys. GnRH agonist therapy (e.g. buserelin) restores the expression of genes involved in germ-cell maturation (*NHLH2, PRDMs*, and *PIWI/TDRD* pathways), thereby re-establishing mini-puberty. We argue that early surgery should be complemented by individualized hormonal therapy as a fertility-preserving intervention in a biopsy-selected subset of cryptorchid boys.

Keywords Cryptorchidism, RNA sequencing, GnRHa treatment, Mini-puberty, Infertility

Résumé

En combinant histologie, endocrinologie et transcriptomique, cet article démontre que l'infertilité chez de nombreux garçons cryptorchides découle d'un échec de la mini-puberté plutôt que d'un simple problème de position. Le traitement par agoniste de la GnRH restaure l'expression de gènes clés de la maturation germinale (*NHLH2, PRDM, PIWI/TDRD*) et réactive la physiologie de la mini-puberté. L'auteur préconise une approche personnalisée, guidée par la biopsie, associant chirurgie précoce et traitement hormonal ciblé pour préserver la fertilité.

Mots-clés : Cryptorchidie, Séquençage d'ARN, Analogue du GnRH, Mini puberté, Infertilité

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Introduction

Cryptorchidism is one of the most common congenital disorders among men, and is a leading cause of subfertility and infertility, even after technically successful orchidopexy. Convergent clinical–molecular data have reframed cryptorchidism as being not merely a problem of malposition but rather a disorder of postnatal testicular maturation. Specifically, in cases of cryptorchidism, mini-puberty fails to trigger the gonocyte → Ad (dark) spermatogonia transition, which is the foundational stem-cell event enabling lifelong spermatogenesis. (1- 4) My group and others have demonstrated that the presence of Ad spermatogonia during early childhood predicts normal adult sperm output, whereas their absence portends oligozoospermia/azoospermia, even after timely and anatomically successful surgery [1–4].

Parallel transcriptomic studies from cryptorchid testis biopsies have identified actionable molecular derangements, including blunted expression of genes that govern mini-puberty signaling (e.g. *NHLH2*), *PRDM* histone methyltransferases, *PROK/ EGR/ PITIX* and *PIWI/TUDOR/MAEL/DDX4* transposon-silencing machinery, which can be rescued by GnRH agonist (GnRHa; buserelin) therapy [4–6]. Even when orchidopexy is performed within current guideline windows, adult fertility remains compromised, especially in cases of bilateral cryptorchidism [2]. The historical treatment model was purely surgical: bring the gonad to the scrotum early, to reduce malignancy risk and "protect" spermatogenesis. However, longitudinal follow-up and semen data have revealed a different reality: if mini-puberty has failed, anatomical relocation alone does not reprogram the immature testis [1–3].

Mini-puberty encompasses the transient activation of the hypothalamic–pituitary–gonadal (HPG) axis in males from ~2 weeks to 3–6 months of age, which drives Leydig cell testosterone and Sertoli cell proliferation (AMH and inhibin B) and, crucially, promotes the first postnatal maturational step: transformation of centrally located gonocytes into Ad spermatogonia along the basement membrane [1–3]. Failure of this step leads to truncation of the stem-cell pool that seeds later spermatogenesis; although the testis may look "normal" in size and even descend with surgery, their fertility potential has already been impaired [1–3]. This insight suggests that such cases may benefit from targeted hormonal treatment to reinstate mini-puberty biology.

In this paper, we review the molecular evidence that has accrued since the 1970s and been refined through contemporary transcriptomics, which supports GnRHa

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treatment as a rational disease-modifying therapy in a biopsy-selected subset of cryptorchid boys.

From histology to outcome: Ad spermatogonia as the fertility gatekeeper

The Ad-centric model

In landmark analyses, boys who underwent early orchidopexy and testicular biopsy were followed into adulthood, with semen testing. The presence of Ad spermatogonia (Ad+) at the

time of surgery predicted normal total sperm counts in ~94% of cases, whereas absence of Ad spermatogonia (Ad−) predicted abnormal spermiograms in ~92% of cases, despite technically successful surgery [1, 2]. In subsequent series, Ad spermatogonia status was validated as a discriminating factor for fertility outcome, and a characteristic deficiency of the gonadotropin luteinizing hormone (LH) was highlighted as an endocrine signature of compromised mini puberty in many cryptorchid males [2, 3,4] (Fig. 1).].

Why surgery alone is insufficient

Orchidopexy only corrects position and cannot trigger the gonocytes → Ad transition or restore a missed mini puberty. Both the treatment timing and adequate biological correction are important. Surgery is necessary to minimize the risk of malignancy and mechanical sequelae, and hormonal intervention is also required to modify the germ-cell fate trajectory [1–5].

Etiology: from endocrine milieu to epigenome

Hypothalamic–pituitary signaling and mini-puberty failure

Cryptorchid boys with high infertility risk histology (HIR; Ad−) display molecular signatures of attenuated mini-puberty[2,5,6]. Among genes identified as involved in idiopathic hypogonadotropic hypogonadism and descent biology, nescient helix-loop-helix 2 (*NHLH2*) mRNA is uniquely decreased in HIR biopsies and robustly increased after GnRHa therapy, positioning *NHLH2* as a plausible controller of mini-puberty that may link neuronal (hypothalamic) activity to testicular responses [6].

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In-utero estrogenic environment

Our earlier placental studies demonstrated elevated estradiol in placentas from mothers of cryptorchid boys compared to controls, suggesting that a prenatal hormonal milieu may derail germ-cell programming. [7]. Viral infections (e.g. Zika) have also been associated with cryptorchidism in affected cohorts, and research in this field continues to disentangle infections per se from hormonal/placental pathways [8].

Epigenetic resetting and transposon control

In primordial germ cells, epigenome erasure and re-establishment are orchestrated by *PRDM* family histone methyltransferases and master regulators (e.g. *SOX17* and *PRDM1/BLIMP1*). HIR cryptorchid testes exhibit downregulated *PRDM* transcripts and pluripotency/PGC programs, which increase after GnRHa treatment [5]. Concomitantly, components of the PIWI–*TUDOR–MAEL–DDX4–GTSF1* axis—which silence retrotransposons and protect genome integrity in the germ line—are impaired in HIR cryptorchid testes and partly restored with therapy [9]. Overall, these data suggest that hormonal rescue occurs not only due to steroidogenesis but also via epigenome repair and reactivation of genome defense.

Molecular reversibility with hormonal treatment

GnRHa restores mini-puberty programs

Transcriptome-profiled biopsies of HIR cryptorchid testes reveal that administration of GnRHa (buserelin) for ~6 months induces broad changes in genes involved in hormonal response, steroidogenesis, and Sertoli–Leydig crosstalk [2,5,]. The unique up-regulation of *NHLH2* following treatment suggests that the neuronal GnRH pathway entrains testicular gene networks, constituting a bridge between central pulsatility and peripheral germ-cell maturation [6].

*PRDM*s and epigenetic remodeling

PRDM family members (e.g. *PRDM9*) mark recombination hotspots and, more broadly, coordinate chromatin states. HIR cryptorchid testes exhibit downregulated *PRDM* mRNAs, which rise after GnRHa therapy—providing molecular evidence that treatment

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re-engages histone methylation programs that are germane to stem-cell identity and meiosis readiness [5].

Long non-coding RNAs and network stabilization

In HIR cryptorchid testes, long non-coding RNA (lncRNA) expressions are perturbed, and shifts toward Ad+-like profiles after GnRHa treatment. Notably, GnRHa therapy leads to upregulation of *BOD1L2*, a candidate participant in the maintenance of spermatogonial stem-cell programs [10]. Together with restoration of PIWI pathway components (transposon silencing), these changes imply that hormonal therapy induces network-wide stabilization of the germline transcriptome [10].

Epididymal development and *CFTR* signaling

Clinically, compared to surgery alone, successful hormonal–surgical management is associated with more normal epididymal development. [11].Molecular work from our group suggests that GnRHa can influence androgen-sensitive epididymal genes, and that CFTR-related pathways may intersect with LH/fibroblast growth factor (*FGF*) signaling in cryptorchidism-related azoospermia [12,13]. While further research is needed to validate involvement of the *CFTR* axis, the initial data align with epididymal pathologies observed in inadequately treated cases

Clinical evidence: from biopsy to semen

Biopsy-guided prognosis and therapy

Across cohorts, risk is stratified by Ad spermatogonia status at orchidopexy: Ad+ children have a high likelihood of normal adult semen, while Ad− children carry a high risk of severe spermatogenic failure without additional therapy [1–4]. Importantly, this differentiation is maintained even with early and technically successful orchidopexy [1–4]. These data support the importance of routine testicular biopsy during orchidopexy at specialized centers—both as a prognostic tool and as therapeutic triage for GnRHa [1 4].

Adult outcomes with GnRHa therapy

In our long-term follow-up of HIR (Ad−) boys treated with GnRHa after surgery, the majority exhibited rescued adult fertility, compared with similarly staged untreated

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controls [2,14]. These results are consistent with the molecular re-engagement of mini-puberty programs and germline protection. On the other hand, HIR cases treated with surgery alone exhibited catastrophic sperm counts in adulthood [2,14].

Re-examining etiological threads

Placental estradiol and endocrine disruption

Virus-induced endocrinological effects on male sexual development could explain a potentially critical component of cryptorchidism. This proposed model is consistent with some of the relevant physiological and seasonal data and applies to all viral infections that affect estrogen levels in placental cells. [15].

Neurodevelopmental linkage and “memory genes”

An elevated odds ratio for low IQ has been found for cryptorchid boys. [16,17]. Furthermore, poor school performance has been observed in cryptorchid boys with impaired mini puberty. [16,17]. One unanticipated signal in our datasets is that HIR patients with and without GnRHa treatment show differential expression of neuronal genes linked to memory and cognition [18,19]. Impaired expression of genes, *EGR4, FMR2 (AFF2)* and *VCX3A*,known to encode proteins involved in signaling pathways that regulate cytoskeletal organization, synaptic vesicle transport and the establishment of connections between neuronal cells may contribute to reduced intellectual and cognitive functioning in infertile cryptorchid males. This corresponds with historical observations of LH deficiency (Fig. 1), and with reported cognitive differences in cohorts that exhibit HPG axis disruption in infancy. The shared genetic toolkit of the brain and testis, along with the dual neuronal–testicular role of *NHLH2*, offer a coherent biological framework for the systemic reach of mini-puberty and its failure [19]. GnRHa treatment augments LH and testosterone secretion and induces testicular expression of various genes involved in long-term memory formation, in particular *RASGRF1* and *EGR2*. [19]. *RASGRF1* plays a key role in regulating the RAS signaling pathway and is important for long-term memory formation. [19]. No positive effect is detectable in “surgery only” patients [19].

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Practical algorithm: who should receive hormonal treatment

1. All boys with undescended testes and not responding to the hormonal treatment should undergo timely orchidopexy, per guidelines.
2. Trained teams should perform intraoperative biopsy, including standardized counting of Ad spermatogonia and germ cells/tubule (fertility index).
3. For Ad+ patients with adequate germ cell counts, surgery alone is often sufficient.
4. Ad−/HIR histology patients should be offered treatment with GnRHa (buserelin) on a curative schedule (~6 months), ideally within an age window during which plasticity is maximal. These patients should be monitored for hormonal markers and, where feasible, molecular response.
5. Follow-up should be performed until adolescence/adulthood—including testicular volume, endocrine axis assessment, and semen analysis where appropriate.

This biopsy-guided approach does not mean that every patient should receive hormone treatment, but rather involves precision medicine grounded in histology and molecular reversibility.

Addressing common objections

- "Surgery is enough, if done early." Early surgery is essential but is insufficient when Ad spermatogonia are absent. In multiple cohorts, long-term follow-up has revealed persistent infertility risk despite timely orchidopexy. [1,20,21]. Impaired transformation of gonocytes into Ad spermatogonia is not the result of temperature stress but rather of a severe hormonal imbalance [22].
- "Hormonal therapy only transiently moves testes." The objective of hormonal treatment here is not positional correction but rather the re-instatement of mini-puberty programs. Molecular evidence for this treatment is provided by observations of GnRHa-induced transcriptomic reversals of *NHLH2, PRDMs, PIWI/TUDOR/MAEL/DDX4*, and lncRNAs
- "Safety/efficacy uncertainties?" GnRHa has a long safety record for use in pediatric endocrinology. In cryptorchidism, its benefits are anchored in histology-linked selection and objective adult semen end-points recorded in

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observational cohorts; randomized trials are challenging but increasingly feasible via multicenter networks

Conclusions

The last half-century of cryptorchidism research has overturned the notion that outcome is dictated by position alone. The decisive variable is whether the testes have experienced the mini-puberty program and completed the gonocytes → Ad spermatogonia transition. Biopsy-proven absence of Ad spermatogonia predicts adult infertility, regardless of early surgical success. However, crucially, molecular evidence shows that this state is reversible with GnRHa treatment. The post-therapy restoration of *NHLH2, PRDMs, EGRs* and *PIWI/TUDOR/MAEL/DDX4/GTSF1* pathways, and reparative lncRNA shifts provides strong biological and clinical evidence justifying hormonal treatment for selected cryptorchid boys. If the goal is fertility preservation, "surgery-only" paradigms should be replaced with routine biopsy-guided decision-making and early endocrine–surgical collaboration. Based on the presently available evidence, hormonal treatment, when judiciously applied to biopsy-selected patients, is not an embellishment—it is pathophysiology-congruent, fertility-preserving therapy.

Abbreviations

CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; DDX4/25: DEAD-Box Helicase 4/25; DMRTC2: DMRT-Like Family C2; FGFR1: Fibroblast Growth Factor Receptor 1HIR: High infertility risk group. NHLH1/2 Nescient helix loop helix ;PIWIL 1–4: Piwi-Like RNA-Mediated Gene Silencing 1–4

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008. All aspects of this study were approved by the Institutional Review Board and the Independent Ethics Committee of Vilnius University. Approval was also provided for research involving the use of material (data records or biopsy specimens) that had been collected for non-research purposes (Vilnius Regional Biomedical Research Ethics Committee, No. 158200-580-PPI-17, 11 June 2013).

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

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d) Competing interests Author declares that he has no competing interests

e) Funding none

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Fig. 1 Lower luteinizing hormone (LH) levels in first morning void urine from prepubertal cryptorchid boys. Triangles: Healthy control boys. Blue circles: Unilateral cryptorchid boys. White circles: Bilateral cryptorchid boys. Pediatric Urology Eds Retick A, Cukier J Williams & Wilkins 1987. p 271.

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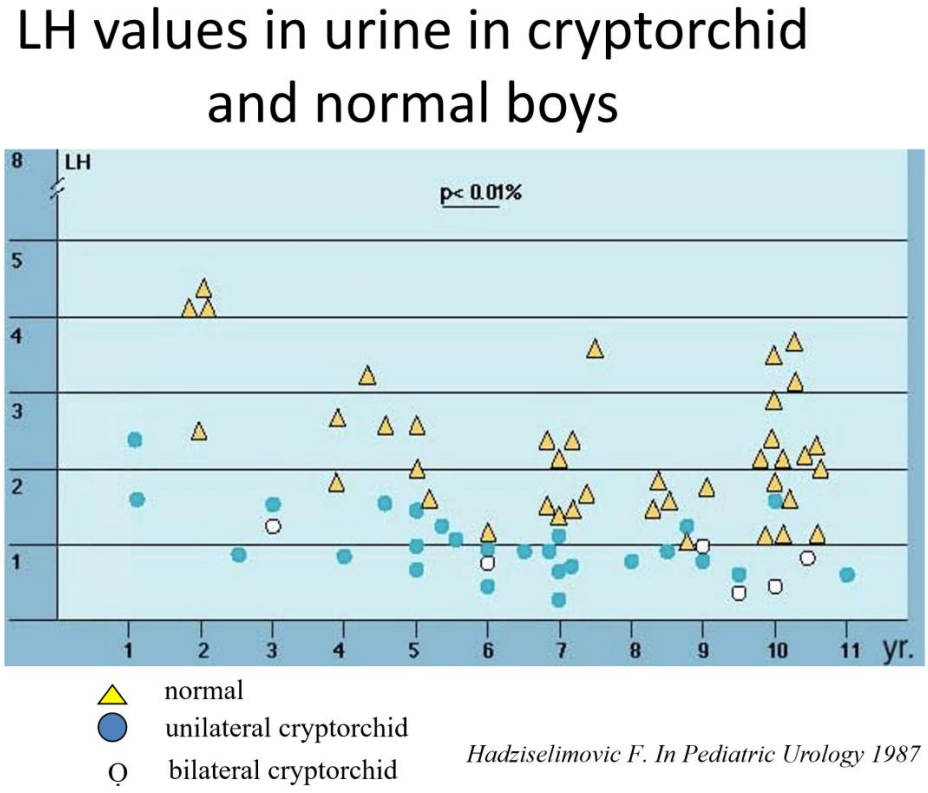

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The role of the Gubernaculum in Testicular Migration – Translational Aspects applied to Undescdnded Testis

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ABSTRACT

Background. Testicular descent is a complex, two-stage developmental program that brings the fetal testis from the abdomen into the scrotum. Central to this process is the gubernaculum testis, a mesenchymal, ligament-like structure that grows, swells, remodels, and ultimately regresses as the testis completes its migration. Failures anywhere along this pipeline manifest as cryptorchidism, with downstream risks for subfertility and malignancy. Although hormones (INSL3, testosterone), the genitofemoral nerve (GFN)/CGRP axis, intra-abdominal pressure, and the processus vaginalis have been studied for decades, a translational synthesis focused on gubernacular structure–function and its practical consequences for pediatric urology is timely.
Objective: To describe the anatomy, histomorphometry, biochemistry, innervation, and vascular anatomy of the gubernaculum and its attachments — translating these insights into operative decision-making (orchiopexy, Fowler–Stephens strategy) and into the interpretation of clinical heterogeneity (ascended testis, ectopias, Prune-Belly syndrome (PBS).

Methods. In this narrative review we showed some topics on: (A) fetal dissection series mapping migration chronology and distal gubernacular insertions; (B) morphometric/ultrastructural analyses of the gubernacular matrix in normal fetuses, undescended/ascended testis, and PBS; (C) arterial supply to the fetal testis during descent; and (D) classification and prevalence of epididymal anomalies relevant to obstructive infertility. Findings are contextualized with seminal work on endocrine and neural regulation of descent and with the conference transcript guiding the present talk.

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Results. The gubernaculum undergoes stage-specific remodeling: early hydration and GAG richness (facilitating swelling and guidance), then a progressive cellular rarefaction occurs as the testis reaches the scrotum. Distal insertions show meaningful variation, including rare perineal, femoral, contralateral scrotal, and pubopenile attachments that explain diverse testicular ectopias; asymmetric migration is uncommon but real. The fetal testis has multiple arterial inputs (testicular, deferential, cremasteric) in the majority of specimens, supporting Fowler–Stephens testicular mobilization when necessary. Epididymal anomalies — disjunction at head/tail or total separation — occur in a clinically relevant minority, particularly in bilateral UDT, and can underlie obstructive infertility despite technically successful orchiopexy.

Conclusions. The gubernaculum is not a passive tether but the anatomical driver and interpreter of multiple signals orchestrating descent. Understanding its microstructure, insertions, innervation, and vascular context sharpens surgical planning, explains hormonal treatment variability, and refines prognostication. Translational adoption of these principles can reduce redo operations, anticipate ectopias, and inform fertility counseling.

Key words Gubernaculum, testicular descent cryptorchidism

Résumé

Contexte. La descente testiculaire est un processus développemental en deux étapes guidant le testicule fœtal de l'abdomen vers le scrotum. Le gubernaculum testis, structure mésenchymateuse de type ligamentaire, croît, se tuméfie, se remanie puis régresse à mesure que la migration s'achève. Toute défaillance de cette séquence conduit à une cryptorchidie, avec risque ultérieur de subfertilité et de malignité. Malgré des décennies de travaux sur les hormones (INSL3, testostérone), le nerf génitofémoral (GFN)/CGRP, la pression intra-abdominale et le processus vaginal, une synthèse translationnelle centrée sur la structure–fonction du gubernaculum et ses implications pour l'urologie pédiatrique est pertinente.

Objectif. Décrire l'anatomie, l'histomorphométrie, la biochimie, l'innervation et la vascularisation du gubernaculum et de ses insertions, et traduire ces connaissances en principes opératoires (orchidopexie, stratégie de Fowler–Stephens) et en compréhension de l'hétérogénéité clinique (testicule ascensionné, ectopies, syndrome de Prune-Belly).

Méthodes. Revue narrative portant sur : (A) des dissections fœtales cartographiant la chronologie migratoire et les insertions distales ; (B) l'analyse

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morphométrique et ultrastructurale du gubernaculum dans des fœtus normaux, cryptorchides/ascensionnés et PBS ; (C) la vascularisation artérielle du testicule fœtal ; et (D) la classification des anomalies épididymaires pertinentes pour l'infertilité obstructive.

Résultats. Le gubernaculum présente un remodelage séquentiel : hydratation précoce et richesse en GAG facilitant le gonflement et le guidage, puis raréfaction cellulaire avec l'arrivée du testicule au scrotum. Les insertions distales varient, incluant des fixations périnéales, fémorales, controlatérales ou pubopéniennes expliquant diverses ectopies. La majorité des spécimens montrent plusieurs apports artériels (testiculaire, déférentiel, crémastérique), soutenant l'approche Fowler–Stephens en cas de besoin. Les anomalies épididymaires, plus fréquentes en cryptorchidie bilatérale, peuvent expliquer une infertilité obstructive malgré une orchidopexie techniquement réussie.

Conclusions. Le gubernaculum n'est pas un simple « lien » passif mais l'acteur anatomique intégrant les signaux orchestrant la descente. Sa microstructure, ses insertions, son innervation et son contexte vasculaire éclairent la planification chirurgicale, la variabilité des réponses hormonales et le pronostic. Leur adoption translationnelle peut réduire les réinterventions, anticiper les ectopies et améliorer le conseil en fertilité.

Mots-clés: Gubernaculum, descente testiculaire, cryptorchidie

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Introduction: From Concept to Clinic

The Testicular migration has two morphologically and mechanistically distinct phases: the transabdominal phase (≈8–15 weeks post-conception), and the inguinoscrotal phase (≈15–35 weeks). The first is INSL3-dependent with androgen-mediated regression of the cranial suspensory ligament; the second relies on androgen-sensitized GFN release of CGRP to guide the gubernaculum across the inguinal canal toward the scrotum [1-9]. The gubernaculum is thus the effector organ of descent: it swells, migrates, contracts, and then remodels into a fibrous remnant after the testis arrives [1,5,10-12].

Defects in any component — endocrine, neural, mechanical, or structural — can prevent descent, producing cryptorchidism (UDT) or ectopic positions (perineal, femoral, pubopenile, contralateral scrotum). While endocrine models often dominate discussions, anatomical diversity of the gubernaculum explains many "outlier" presentations and some failures of hormonal therapies [1,5-7,11-15].

This paper emphasizes the gubernaculum-centric view, grounded in our human fetal and pediatric surgical research, to derive clear translational messages for everyday management.

Human Fetal Migration: Chronology and Asymmetry Developmental timing

Large dissection series confirm the canonical timeline: by the late second trimester, the testis approaches or enters the internal ring; by >35 weeks, most fetuses have scrotal testes. In our material, we observed that asymmetric migration is uncommon — only a handful among more than a thousand fetal sides — underscoring a robust bilateral program with occasional side-specific delays [1].

Functional determinants

While hormonal cues (INSL3, testosterone) and GFN–CGRP signaling coordinate the two phases, mechanical elements — intra-abdominal pressure, the evolving processus vaginalis, and the gubernacular cone — provide the structural path and motive dynamics[2,5,8-13]. Experimental disruption of abdominal wall integrity compromises migration; conversely, gubernacular swelling and canal formation are prerequisite to safe passage[1,5,10].

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The Gubernaculum as Organ: Structure, Insertions, Remodeling

Anatomy

During the fetal period, the gubernaculum appears as an elongated, cylindrical mesenchymal structure attaching proximally to the testis/epididymis and distally to inguinal canal **(Figure 1).** Histology shows high cellularity, hydrated matrix, and abundant glycosaminoglycans (GAGs) early on, transitioning to dense collagen and reduced cellularity as descent completes [1,11,12]. Classic biochemical studies corroborate this hydration–dehydration cycle, where GAG-rich swelling is replaced by collagen-dominated consolidation once the testis is scrotal [1,11,12].

Translational take-home. A pliable, hydrated gubernaculum appears conducive to movement; a fibrotic, collagen-dense remnant indicates descent completion or arrested remodeling in UDT and may limit the success of traction-only maneuvers during orchiopexy [1,11,12].

Distal insertions and ectopias

Most gubernacula end in the scrotal region, but variants exist: perineal, femoral, pubopenile, contralateral scrotal, and even abdominal wall insertions. We have documented rare pubopenile terminations (≈2 cases in large fetal series), and we routinely see multiple distal slips in select specimens [1]. These aberrant insertions explain ectopic testes: the testis faithfully follows its gubernaculum. Recognizing this anatomy pre-operatively (ultrasound/MRI in difficult cases) or intra-operatively helps surgeons predict and locate ectopias, plan incision placement, and avoid blind groin exploration.

Translational take-home. When imaging or examination suggests non-palpable or ectopic positions, consider variant distal insertions; map and follow the gubernacular fibers, not only the vas and vessels.

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Proximal relationships: testis–epididymis–gubernaculum

The gubernaculum inserts in conjunction with the epididymis and testicular tunics (**Figure 2**). In normal fetuses, attachments are consistent; in cryptorchidism in almost 35% of the cases we can observe epididymal disjunctions anomalies (head-only, tail-only, or total disjunction) [7,13-15]. Such epididymal anomalies portend post-operative obstructive infertility despite proper testicular positioning.

Translational take-home. At orchiopexy, document epididymal continuity (head and tail). If total disjunction or tail disjunction is present, counsel families about potential obstructive risks and consider tailored follow-up (spermatic obstruction work-up later in life). Carefully preserve and align the epididymal/testicular unit during fixation.

Innervation and the GFN–CGRP Axis: Making the Gubernaculum Move

The inguinoscrotal phase requires more than passive growth: it involves directional migration and contraction of the gubernaculum. A large body of work from Hutson and colleagues and others demonstrated that androgens act, at least in part, via the genitofemoral nerve (GFN) to stimulate release of calcitonin gene–related peptide (CGRP), which in turn induces gubernacular contractions and processus vaginalis dynamics (8–10,13). Denervation experiments in rodents delay descent; antagonism of CGRP alters timing; and exogenous CGRP can modify gubernacular behavior [8,9,13].

Our recent review of gubernacular innervation summarizes human and animal data pointing to rich peptidergic content (including CGRP) inside the gubernacular core and at the distal cone, supporting a model in which neural signals tune matrix hydration and smooth-muscle-like activity within the gubernaculum (5).

Translational take-home. Endocrine therapies that improve androgen tone may aid inguinoscrotal progression only when innervation and gubernacular architecture are intact; if the distal insertion is aberrant or the gubernaculum is fibrotic, endocrine therapy alone will not relocate the testis — a frequent explanation for “hormone failures.” [1,5,8-10,13].

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Matrix Biology: GAGs, Collagen, and Elastic Fibers

The **biomechanics** of the gubernaculum arise from its **extracellular matrix**:

- **Glycosaminoglycans (GAGs).** Early gubernacula are GAG-rich (e.g., dermatan sulfate, hyaluronan), capturing water and enabling swelling and lubricity during migration. As descent completes, GAG content falls, water is lost, and dry mass collagen rises [11,12].
- **Collagen and elastic fibers.** In normal fetal gubernacula, elastic fibers concentrate at the distal cone, while collagen progressively increases during late descent and after the testis is scrotal [1]. In cryptorchidism, gubernacula are often more fibrous with altered collagen organization and GAG profiles, compatible with reduced compliance [1,6,11,12].
- **Prune-Belly syndrome (PBS).** In PBS fetuses, we measured quantitative shifts toward collagen III predominance and altered elastic fiber content within the gubernaculum versus controls, consistent with the syndrome's abdominal wall deficiency and chronic pressure derangements [6].

Translational take-home. A stiff, collagen-rich gubernaculum may restrict safe lengthening during orchiopexy; surgeons should expect dense tissue planes, consider liberating distal slips broadly, and avoid undue traction on the spermatic cord.

Processus Vaginalis, Intra-abdominal Pressure, and Canal Dynamics

The processus vaginalis forms as the gubernaculum invaginates the peritoneum through the inguinal region, creating the inguinal canal before testicular passage. The gubernaculum's swell and pull are essential for this morphogenesis; inadequate invagination links to hernia, hydrocele, and ascending testis [2.8-10,13]. Intra-abdominal pressure supplements the mechanical drive; its perturbation (e.g., in PBS) contributes to failed descent [6,10,16].

Translational take-home. In boys with hernia/hydrocele and UDT, consider the shared pathophysiology: both may stem from gubernacular–

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processus dysmorphogenesis influenced by the GFN–CGRP–androgen axis [9,10,13,16].

Vascular Considerations: Why Fowler–Stephens Works

Our corrosion-cast and microdissection studies in human fetuses established that the testis is supplied by multiple arteries during migration: the testicular artery and deferential artery are constant, with the cremasteric artery present in a majority; two-artery patterns occur, but three-artery or even richer networks predominate (3,4). These findings corroborate adult and pediatric observations that collateral flow can sustain the testis after testicular artery division, providing robust anatomic support for one- or two-stage Fowler–Stephens orchiopexy when primary cord length is inadequate.

Translational take-home. With high intra-abdominal testes, arterial division—judiciously executed—rests on sound fetal anatomic precedent; preoperative planning should still weigh vessel caliber, collateral quality, and the surgeon's experience.

Epididymal Anomalies: The Hidden Link to Obstruction

Anatomical disjunction between the epididymal head/tail and testis (partial or total) has been cataloged since our early fetal studies and is more prevalent in bilateral UDT than unilateral cases [7,14,15]. Contemporary pediatric series confirm a ~20% prevalence of epididymal anomalies with potential spermatic obstruction later in life, not reliably predicted by age, testis position, or patency of the processus vaginalis [15,17,18]. Because the gubernaculum anchors near the epididymal tail as well as testis, anomalous configurations may alter tension vectors and migration mechanics.

Translational take-home. Surgeons should classify the epididymal–testicular relationship intra-operatively, preserve delicate connections, and counsel families about fertility follow-up when significant disjunction or epididymal atresia is present (7,14,15). [7,14,15].

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Special Context: Prune-Belly Syndrome (PBS)

PBS couples abdominal wall aplasia, urinary tract malformations, and bilateral cryptorchidism. In our comparative fetal analyses, the gubernaculum in PBS showed measurable differences in collagen and elastic fiber composition — increased collagen III fraction with fewer elastic fibers — versus controls, without major differences in nerve density (6). Given PBS's chronically altered intra-abdominal pressure and urinary tract distention, these matrix shifts likely degrade the mechanical behavior of the gubernaculum, compounding descent failure.

Translational take-home. Expect stiffer gubernacula and shorter effective length in PBS; anticipate challenging mobilization, a higher likelihood of staged approaches, and the need for collateral-friendly strategies (e.g., Fowler–Stephens).

Why Hormonal Therapy Sometimes "Fails": An Anatomical Answer

Hormonal treatments target endocrine drivers (INSL3/testosterone; indirectly GFN–CGRP) and can aid descent only if the gubernacular architecture and distal target are suitable. In cases where we identify aberrant distal insertion (e.g., perineal, femoral) or advanced collagenization of the gubernaculum, medical therapy predictably yields limited positional change, even if endocrine markers improve [1,5,8-10.13].

Translational take-home. Incorporate anatomical probability into counseling. If the testis is ectopic (e.g., perineal) or imaging suggests fibrotic distal bands, prioritize surgical correction rather than prolonged hormonal trials.

Practical Algorithm for Pediatric Surgeons

1. **Preoperative assessment.** Document palpability, sidedness, and any ectopic trajectory. In non-palpable or atypical cases, consider ultrasound or MRI to map distal gubernacular slips and canal status.
2. **Plan for variability.** Anticipate rare distal insertions; prepare for perineal or femoral exploration if intra-operative cues demand it [1].

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3. **Intra-operative priorities.**
    - Identify and mobilize the gubernaculum broadly; divide restrictive distal bands.
    - Inspect epididymal continuity (head and tail); record anomalies.
    - Respect vascular collaterals; if length is inadequate, stage with Fowler–Stephens leveraging documented deferential/cremasteric inputs [3,4].
4. **When to stage.** High intra-abdominal testis with tight cord and short mesentery → two-stage Fowler–Stephens based on fetal vascular collateral evidence [3,4].
5. **Counseling.** Discuss epididymal anomalies and future obstructive risk; manage expectations about the limited role of hormones when gubernacular anatomy is unfavorable.

Future Directions

- Quantitative imaging of the gubernaculum (elastography, diffusion MRI) to pre-operatively estimate matrix stiffness and hydration, predicting mobilization difficulty.
- Molecular profiling of the human gubernaculum across gestation, integrating innervation markers (CGRP, neurofilaments) with matrix genes to define therapeutic windows.
- Prospective registries linking epididymal anomaly class at orchiopexy to adolescent/adult semen outcomes to refine fertility risk stratification.
- PBS-specific pathways: interventional studies on matrix modulation and abdominal wall reconstruction timing to facilitate testicular descent.

Conclusion

The gubernaculum testis remains the keystone organ of testicular descent. Its architecture, insertions, innervation, and matrix remodeling determine not just whether the testis descends, but where it goes and how it can be brought down safely when it does not. Translating these anatomical truths into pediatric urology sharpens our operative strategies (especially for high and ectopic testes), clarifies the limits of hormonal therapy, and illuminates

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persistent fertility questions through the lens of epididymal–gubernacular anatomy. Integrating fetal-anatomic knowledge into modern care is not an academic luxury — it is a practical necessity for better outcomes.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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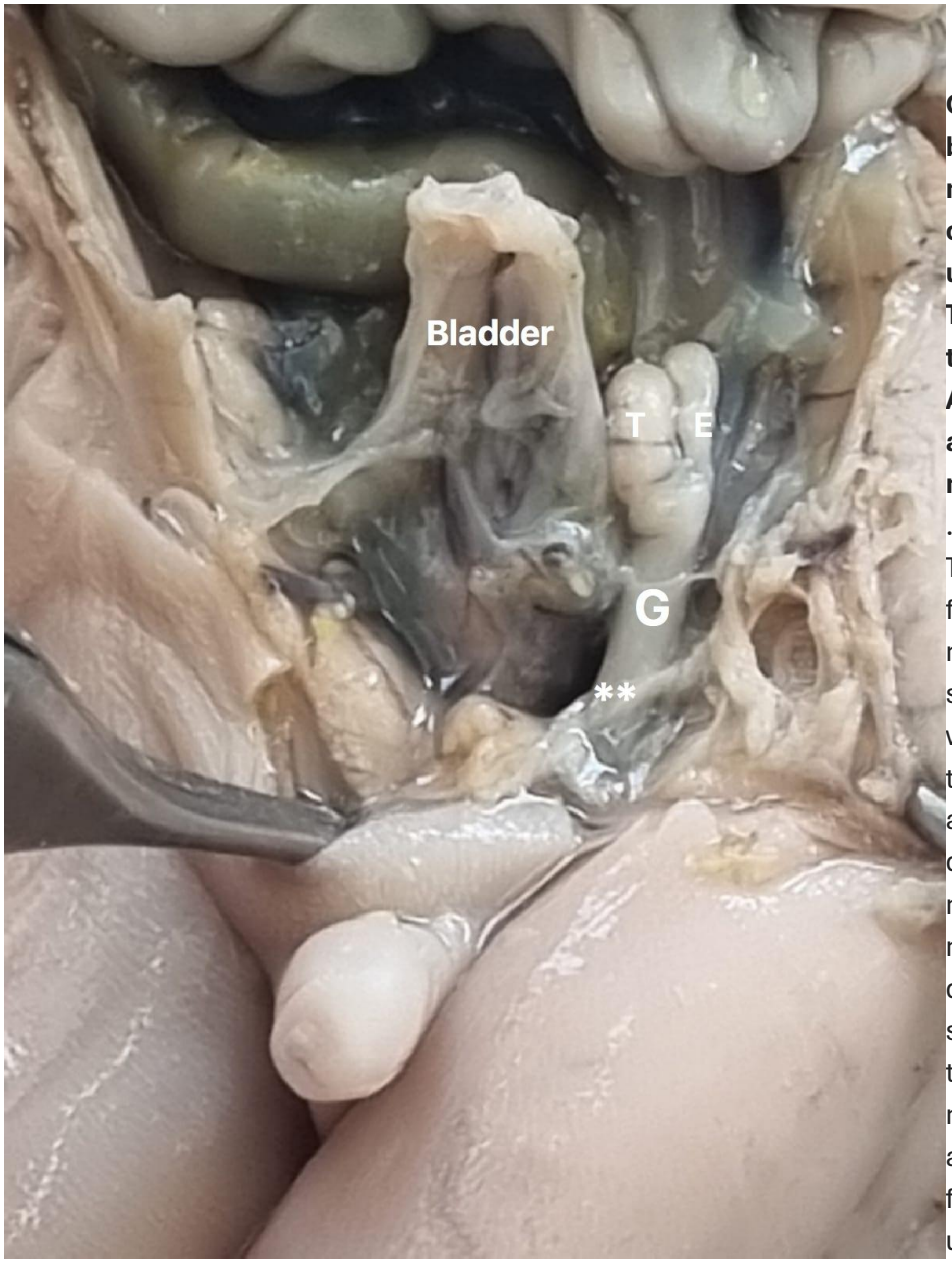
Figures:

Figure 1:



Gubernaculum Testis Anatomy. The figure shows the abdominal dissection of a fetus with 20 weeks post-conception. We can observe the gubernaculum testis (G) in intra-abdominal position. T- Testis, E – Epididymis and * - Internal inguinal Ring. [1].

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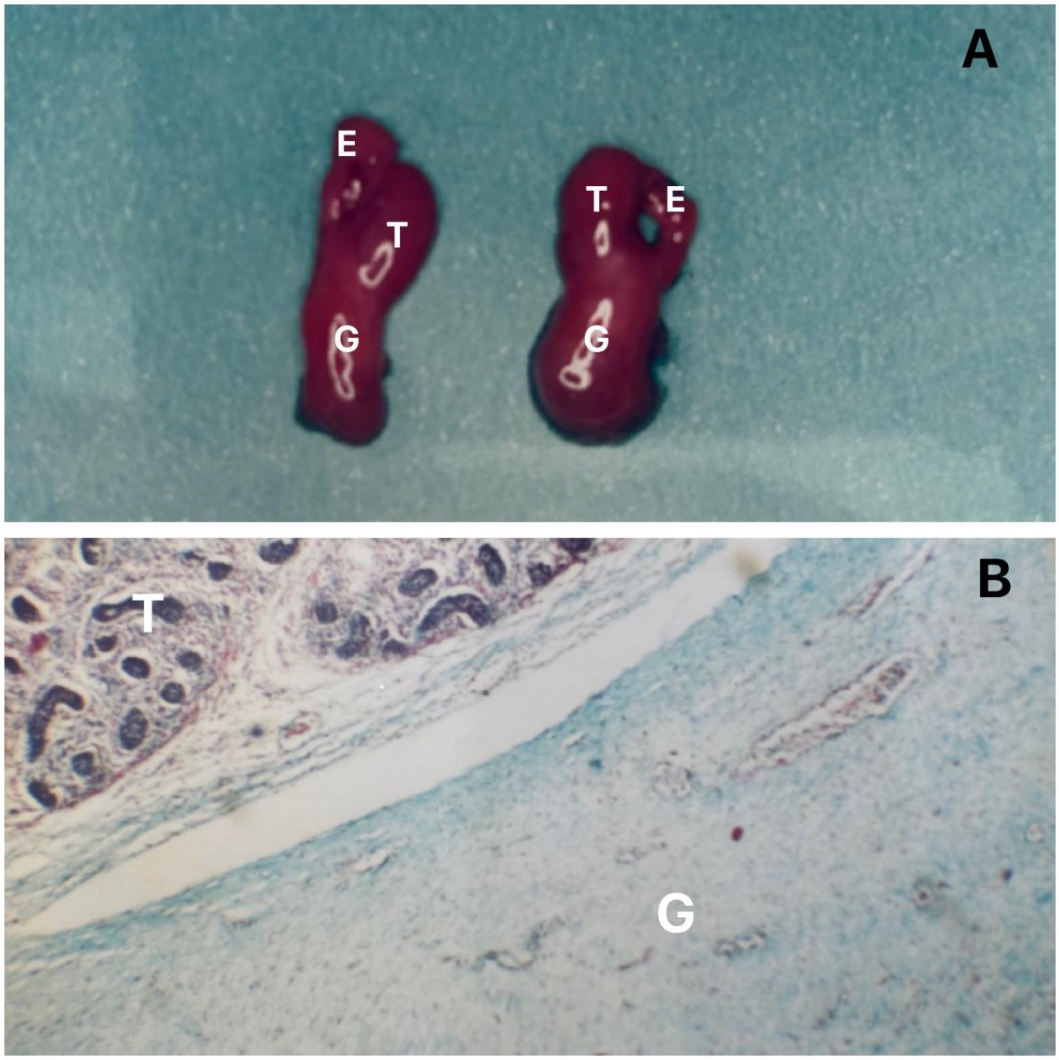
Figure 2:



Proximal insertion of gubernaculum testis. A) The testis and gubernaculum (G) were dissected and we can observe the relationship between the gubernaculum proximal insertion with testis (T) and epididymis (E) and B) Photomicrography of gubernaculum proximal insertion. We can observe the relationship between the Tetis (T) and gubernaculum. Trichromic of Masson X40.

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Pitfalls in cryptorchidism diagnosis

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Abstract

Accurate clinical distinction among undescended, ectopic, retractile and acquired/ascending testes underpins every subsequent decision in cryptorchidism. Misclassification drives both over- and under-treatment, with repercussions for fertility preservation, malignancy surveillance, resource use, and family counseling. This paper synthesizes practical pitfalls encountered from referral to definitive management, aligning bedside examination with contemporary guideline recommendations and translational insights. Drawing from our clinical program in Novi Sad and collaborative work with Faruk Hadžićselimović and colleagues, we emphasize five recurring traps: (1) equating any "empty" hemiscrotum with an undescended testis; (2) over-reliance on ultrasonography for palpable testes or to "rule in" non-palpable testes; (3) under-recognition of acquired/ascending testes and retractile physiology; (4) treating heterogeneous risk categories as homogeneous—particularly when biopsy interpretation is unavailable or suboptimal; and (5) ignoring environmental and systemic contexts that modulate risk and presentation. We propose a streamlined diagnostic pathway that privileges meticulous physical examination, judicious use of laparoscopy for non-palpable testes, selective adjunct testing, and context-aware counseling. Throughout, we integrate histological and molecular evidence (e.g., adult-dark [Ad] spermatogonia as a prognostic marker) to show how apparently "simple" diagnostic slips alter life-course outcomes.

Key words Cryptorchidism, pitfalls, treatment

Résumé

Une distinction clinique précise entre testicules non descendus, ectopiques, rétractiles et acquis/ascensionnés conditionne toutes les décisions ultérieures en matière de cryptorchidie. Les erreurs de classification entraînent à la fois surtraitements et sous-traitements, avec des conséquences sur la préservation de la fertilité, la surveillance oncologique, l'utilisation des ressources et l'information des familles. Cette synthèse décrit les écueils pratiques rencontrés depuis l'orientation jusqu'à la prise en charge définitive, en alignant l'examen clinique bedside avec les recommandations contemporaines et les données translationnelles.

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S'appuyant sur notre programme clinique à Novi Sad et sur les collaborations avec Faruk Hadžićselimović et collègues, nous identifions cinq pièges récurrents : (1) assimiler tout hémiscrotum « vide » à un testicule non descendu ; (2) surestimer l'utilité de l'échographie pour les testicules palpables ou pour « confirmer » un testicule non palpable ; (3) méconnaître les testicules acquis/ascensionnés et la physiologie rétractile ; (4) traiter des catégories de risque hétérogènes comme homogènes, surtout lorsque la biopsie est indisponible ou mal interprétée ; et (5) ignorer les contextes environnementaux et systémiques modulant les risques et les présentations.

Nous proposons un algorithme diagnostique rationalisé privilégiant un examen physique minutieux, une utilisation judicieuse de la laparoscopie pour les testicules non palpables, des examens complémentaires ciblés et un conseil contextualisé. L'intégration de données histologiques et moléculaires—notamment la valeur pronostique des spermatogonies adultes sombres (Ad)—montre comment des erreurs diagnostiques apparemment simples peuvent infléchir les trajectoires de vie.

Mots-clés: Cryptorchidie, pièges, traitement

Introduction: why diagnosis (still) matters

For many trainees, orchiopexy is introduced as a "straightforward" pediatric case. Yet the clinical reality is knotty: the same child can be scheduled for surgery in one center and discharged with reassurance in another. That variability often reflects diagnostic pitfalls rather than genuine epidemiology. The consequences are not trivial. Perform an unnecessary orchiopexy on a retractile testis and you subject a child to anesthesia and create scar without benefit; miss an undescended or ascending testis and you delay the only intervention shown to optimize fertility potential and reduce malignancy risk when performed early. Contemporary guidelines from both the American Urological Association (AUA) and the European Association of Urology (EAU) explicitly anchor management in accurate, exam-based classification, discourage pre-referral imaging, and recommend early orchiopexy for truly undescended testes—ideally by 12 months and no later than 18 months of age [1,2]. The rationale is not cosmetic: timely descent (surgical or, in selected contexts, hormonal) intersects with mini-puberty biology and the maturation of Ad spermatogonia, a determinant of adult spermatogenic capacity [3,4].

This paper is deliberately pragmatic. It is written from the perspective of a clinician who has stood at the operating table only to discover both testes in the scrotum of a child listed for "bilateral cryptorchidism," and who has also seen non-palpable testes missed until school age—when salvage of fertility potential is less certain. It translates those lived clinic and operating-room tensions into five domains where diagnosis most commonly goes wrong.

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Definitions and the taxonomy trap

What we must call things (and why words matter)

Undescended testis (UDT) is an umbrella term encompassing *true undescended* (arrested along the normal path from abdomen to scrotum) and *ectopic* (deviated off the normal path). **Retractile testis** is a *normally descended* testis that can be manipulated to the scrotum and *remain* there without tension after cremasteric relaxation; it often rides high transiently due to an exuberant cremasteric reflex. **Ascending (acquired) testis** describes a testis documented in the scrotum earlier that later resides persistently supra-scrotal, likely due to an inelastic processus vaginalis or differential somatic growth. Finally, **non-palpable testis** includes intra-abdominal testes, canalicular testes hidden by body habitus, vanishing testes, or agenesis.

Failure to apply these labels rigorously begets algorithmic error. “Empty hemiscrotum → ultrasound → surgery” is a frequent but flawed chain. Pre-referral imaging is specifically discouraged by AUA/Choosing Wisely and international guidelines because it rarely changes management, can be misleading, and delays definitive care [1,2]. The diagnosis of cryptorchidism is, first and foremost, clinical.

Pitfall #1 — Mistaking retractile for undescended testis (and vice versa)

Why the exam beats the scan

A careful physical examination, performed in a warm room, with patience for cremasteric relaxation, and with the non-dominant hand sweeping from the anterior superior iliac spine along the inguinal canal, remains the most powerful diagnostic tool we have. In ~70% of referrals for “undescended testis,” a testis is palpable, and when palpable, management is largely determined without imaging [2]. Ultrasonography can “make” a retractile testis undescended, and it can’t reliably exclude a non-palpable testis; even in experienced hands, the testis may be missed in the canal or mistaken for lymph node or bowel. This is why AUA and EAU recommend against routine ultrasound prior to referral or surgery for palpable testes and against CT/MRI altogether [1,2].

Consequences of misclassification

Retractile testes do **not** require orchiopexy and, when truly retractile, carry fertility outcomes comparable to controls if left alone and monitored [6]. Conflating retractile with undescended drives unnecessary operations and parental anxiety. Conversely, labeling a true UDT as “retractile” delays orchiopexy beyond the window where germ cell maturation can be optimized (by ~12 months), risking poorer spermatogenic outcomes later in life [2–4].

Practical tips to avoid the trap

- Examine with distraction and warmth; repeat after the child settles, preferably with child’s legs in frog position.

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- Fatigue the cremaster: hold the testis gently in the scrotum for 30–60 seconds; a retractile testis tends to "stay."
- Document position precisely (e.g., **high-scrotal**, **superficial inguinal pouch**, **canalicular**, **peeping**, **non-palpable**).
- Re-examine across visits—retractiles can be brisk early in childhood and "calm" later.
- When in doubt in older boys with a high-riding testis that will not remain scrotal, consider the possibility of acquired/ascending testis, not "retractile forever."

Pitfall #2 — Over-reliance on ultrasonography and other imaging

What the evidence and guidelines actually say

Beyond operator variability, ultrasonography has limited sensitivity for localizing non-palpable testes and does not change the operative plan: if the testis is non-palpable, diagnostic laparoscopy both localizes and treats. Professional societies explicitly advise **against** ordering ultrasound, CT, or MRI for palpable or non-palpable testes during initial evaluation, because imaging rarely improves accuracy and often delays definitive management [1]. The EAU guideline reaches the same conclusion and prioritizes laparoscopy for non-palpable testes [2].

Why scans persist (and how to move forward)

Common drivers include parental expectation ("We need a scan to be sure"), institutional habit, and referring-physician discomfort. Clear communication helps: family value prompt, confident, hands-on assessment and an explanation that laparoscopy is the most accurate—and often therapeutic—test when a testis is truly non-palpable [2].

Pitfall #3 — Missing acquired/ascending testes

An important subset of boys present with a history of previously scrotal testes that "ride up" over time. These *acquired* or *ascending* testes are not benign variants; their environmental and anatomical underpinnings differ from congenital UDT, but they still merit orchiopexy once ascent is established. Failing to recognize ascent—especially when earlier documentation exists—postpones repair and may erode fertility potential. Vigilant well-child examinations and accurate documentation of testis position at each pediatric visit are crucial; the AUA guideline underscores timely referral once ascent is identified [1].

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Pitfall #4 — Treating heterogeneous risk the same (the biopsy question)

Why histology matters (if you can get it right)

Seminal work has shown that the presence or absence of Ad spermatogonia at orchiopexy correlates with later spermatogenic capacity; boys lacking Ad spermatogonia are a *high-infertility-risk* subgroup who may need intensified counseling and, in some protocols, adjuvant therapy [3]. Subsequent series—including national cohorts—have confirmed that a meaningful fraction of boys, even with unilateral UDT, lack Ad spermatogonia at typical ages of repair [3]. These data support the idea that "one size" counseling does not fit all.

The practical barrier

Pathology services must be able to process small testicular biopsies and report beyond "juvenile testis" to germ-cell counts and the presence/absence of Ad spermatogonia. Without such capability, routine biopsy risks being uninformative, adding morbidity without guiding care—precisely the problem many centers face. Our Novi Sad experience mirrors others': unless there is a committed and trained pathologist, biopsies rarely influence decisions (as discussed in our clinical reflections) [8]. When robust histologic reporting is available, biopsy during orchiopexy can stratify risk and refine follow-up; when it is not, biopsy should not be a ritual.

Pitfall #5 — Ignoring the wider context: environment, syndromes, and DSD

Syndromic and DSD flags that must alter the path

Hypospadias, ambiguous genitalia, bilateral non-palpable testes, or atypical genital anatomy warrant coordinated evaluation for differences/disorders of sex development (DSD) with endocrinology and genetics. Here, the *mini-puberty* window (weeks to months after birth) is a unique diagnostic moment to collect gonadotropin and testosterone data before the hypothalamic-pituitary-gonadal axis quiets [4]. A UDT work-up divorced from this context risks missing CHH or other endocrine disorders that change treatment entirely.

Environmental exposures and population differences

Cryptorchidism is a canonical endpoint in the "testicular dysgenesis syndrome" framework, where maternal smoking, certain pesticides and endocrine-disrupting chemicals have been variably associated with risk. While causal inference is complex, clinicians should be aware that regional exposures (including poorly regulated pesticides or post-industrial pollutants) can shape population-level histology and prevalence, as we observed when comparing Serbia with Switzerland and parts of the USA in different eras [6].

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A stepwise, exam-first diagnostic pathway

Step 1. History & context

- Perinatal history, prematurity, prior documentation of scrotal testes, family history.
- Red flags: hypospadias, ambiguous genitalia, bilateral non-palpable testes, syndromic features (e.g., Prader–Willi, Kallmann).

Step 2. Physical examination (the crux)

- Warm room, patient supine and frog-legged; gentle sweeping maneuver.
- Determine **palpable vs non-palpable**, **position**, and **mobility**; classify as **retractile** if it can be manipulated into the scrotum and remains there after cremaster fatigue.

Step 3. Decision node: palpable vs non-palpable

- **Palpable UDT** → orchiopexy planned; no ultrasound needed [1,2].
- **Retractile** → no surgery; annual follow-up to detect possible ascent [1,2].
- **Non-palpable** → diagnostic laparoscopy (gold standard) because it localizes and often treats in one session [2].

Step 4. Adjuncts—used selectively

- **Ultrasonography** only in selected obese children with equivocal canal findings where it meaningfully clarifies preoperative planning—not as a screening test [1,2].
- **Endocrine work-up** in bilateral non-palpable testes, DSD flags, or infants within mini-puberty where CHH or other endocrine etiologies are suspected [4].
- **Testicular biopsy** during orchiopexy only if a dedicated pathology partner can report Ad spermatogonia/germ-cell counts and findings will influence counseling/management [3,5].

Special scenarios where diagnosis goes awry

The chubby toddler with a "non-palpable" testis

Increased adiposity frustrates palpation; this is the rare case where ultrasonography can be pragmatically useful if it shows a canalicular testis that will be addressed via inguinal exploration, but the default remains examination and timely referral—not serial scanning [1].

Bilateral "non-palpable" testes in a newborn

This is an endocrine emergency until proven otherwise; evaluation should occur during mini-puberty to leverage physiologic LH/FSH/testosterone peaks. MRI/CT are not first-line; coordinated endocrine and surgical evaluation is.

"Disappearing" testis (vanishing testis syndrome)

Laparoscopy that reveals blind-ending vessels above the internal ring obviates further groin exploration; avoid the pitfall of "chasing" a testis that involuted antenatally [2].

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The adolescent with a high-riding testis

Do not assume retractile status; acquired ascent is common. Prior documentation of scrotal position transforms the diagnosis to *ascending testis* requiring orchiopexy [1,2].

Translational lens: why timing follows biology

Mini-puberty—a physiologic postnatal surge in gonadotropins and testosterone—promotes transformation of gonocytes into Ad spermatogonia, seeding future spermatogenesis. In cryptorchidism, impaired testicular environment and hormonal signaling jeopardize this transition. Histologic series show that absence of Ad spermatogonia at orchiopexy predicts impaired adult sperm output; conversely, timely intervention aligns with more favorable maturation [3]. This biology is why guidelines press for **early** orchiopexy—**by 12 months** (and no later than 18 months)—and why protracted diagnostic pathways fueled by unnecessary imaging are not benign administrative detours but potential biologic harm [1,2].

Ten practical "anti-pitfall" rules

1. **Name it right:** distinguish undescended, ectopic, retractile, and ascending—precisely.
2. **Trust your hands:** a warm, unhurried exam outperforms routine ultrasound for palpable testes.
3. **Do not image reflexively:** ultrasound/CT/MRI rarely help and often delay; laparoscopy is the diagnostic test for non-palpable testes [1,2].
4. **Refer early:** schedule orchiopexy **by 12 months**, **no later than 18 months** [1,2].
5. **Audit documentation:** record exact position and mobility at every well-child exam to catch ascent early.
6. **Consider the context:** DSD flags or bilateral non-palpable testes mandate endocrine co-management and mini-puberty timing.[4].
7. **Use biopsy wisely:** only where results (Ad spermatogonia, germ-cell counts) will be reported accurately and acted upon [3].
8. **Counsel honestly:** retractile testes need surveillance, not surgery; acquired ascent is real and treatable.
9. **Temper certainty:** when centers disagree widely on histologic "risk mix," look for system artifacts (selection, timing, pathology reporting), not "better surgeons."
10. **Teach complexity:** stop calling cryptorchidism a "basic case." It is a window into developmental endocrinology, onco-fertility, and surgical judgment.

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Conclusions

The *apparent* simplicity of orchiopexy belies the diagnostic nuance required to decide *who* needs it, *when*, and *how* to counsel about future fertility. The most costly mistakes are often made before the first incision: labeling a retractile testis "undescended," scanning instead of examining, or letting a non-palpable testis languish in a queue of imaging studies rather than proceeding to laparoscopy. When we align practice with evidence—exam-first classification, early referral and repair, selective adjunct testing, and center-appropriate use of biopsy—we respect both the biology of mini-puberty and the long-term interests of our patients. Cryptorchidism should be taught and practiced not as a routine, but as a disciplined exercise in developmental diagnosis.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Epididymo-testicular descent

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Abstract

The descent of the male gonad is a coordinated morphogenetic journey of the epididymo-testicular unit, not of the testis in isolation. A century of schematic drawings placed the gubernaculum at center stage, but histology, comparative embryology, and translational studies have indicated that the epididymis precedes and “leads” the testis into the scrotum, with the gubernaculum primarily creating the path and providing space rather than “towing” the testis. This paper synthesizes molecular, anatomical, and clinical evidence supporting this epididymis-first concept and revisits classic experimental data to integrate modern insights on INSL3/RXFP2 signaling and androgen action. I also discuss how dissociation anomalies between the epididymis and testis, as well as a failure of epididymal smooth muscle/myofibroblast maturation, can arrest descent despite apparently intact gubernacular morphology.

Keywords: Gubernaculum, Embryology, Epididymis, Hypogonadotropic hypogonadism, GnRHa

Résumé

La descente du testicule est un processus morphogénétique coordonné de l'unité epididymo-testiculaire, et non un processus isolé du testicule. Pendant un siècle, des schémas ont placé le descente du testicule est un processus morphogénétique coordonné de l'unité épididymo-testiculaire, et non un processus isolé du testicule. Pendant un siècle, des schémas ont placé le gubernaculum au centre de ce processus. Or, l'histologie, l'embryologie comparée et les études translationnelles indiquent que l'épididyme précède et « guide » le testicule dans le scrotum, le gubernaculum jouant principalement un rôle de guide et de création d'espace, plutôt que de tirer le testicule. Dans cet article, je synthétise les données moléculaires, anatomiques et cliniques – y compris les travaux de mon équipe – qui soutiennent ce concept de l'épididyme en premier ; 'intègre les connaissances modernes sur la signalisation

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INSL3/ RXFP2 et l'action des androgènes. J'aborde également la manière dont les anomalies de dissociation entre l'épididyme et le testicule, ainsi que l'échec de la maturation des muscles lisses/myofibroblastes épididymaires, peuvent arrêter la descente malgré une morphologie gubernaculaire apparemment intacte.

Mots clés: Gubernaculum, embryologie, épididyme, GnRHa, Hypogonadisme hypogondotrope

From “gubernacular dogma” to the epididymo-testicular unit

Generations of pediatric surgeons learned that the gubernaculum is the “holy grail” of testicular descent. The narrative, which was popularized by elegant but oversimplified diagrams, envisions a ligament that shortens, pulls, and anchors the testis into the scrotum. However, histology sections, scanning electron microscopy, and cross-species comparisons challenge this picture.

Three biologic observations frame the issue:

1. Among mammals, one never finds “testis down, epididymis up.” In contrast, epididymis down with testis retained intra-abdominally exists (e.g., in chinchilla), implying that epididymal descent can occur independently, whereas testis descent without epididymis does not [1].
2. In fetal and neonatal rodents and humans, the gubernacular mass connects primarily to the epididymis, and in humans it swells to open the inguinal canal rather than functioning as a tensile cable [2-4] (Fig 1,2).
3. Experimental severing of gubernacular components in rats prevents descent when the distal component (future scrotum/processus vaginalis) is cut. This occurs not because a “pulley is severed,” but because the space fails to form. Conversely, descent can proceed when the proximal gubernaculum is cut so long as the scrotum forms, again emphasizing space creation over traction [5].

These observations, updated in my comprehensive review on the epididymo-testicular unit (ETU) [2-4], argue that the epididymis is the active locomotor and steering element of descent, whereas the gubernaculum provides the permissive environment (gelatinous extracellular matrix, expansion of the canal).

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Developmental chronology: who moves first?

Rodent timeline (illustrative)

The cauda epididymis advances ahead of the testis, “bulldozing” the way into the future scrotum; the testis follows, cradled by the everted epididymal tail and mesentery. By postnatal day 3, the ETU is largely scrotal, and the gubernacular “cord” is thin; there is no histologic evidence of strong traction marks on the testis at that stage [2-5]. Testicular form, such as that in human shows flattening of the upper testis pole due to caput epididymis pressure (Fig 1).

Human fetal sections

Human fetal series at 20–30 weeks of gestation exhibited a bulky, hydrated gubernacular cone occupying the inguino-scrotal corridor and was rich in extracellular matrix and fibroblasts. Gubernacular fibers merge into epididymal connective tissue, not into the tunica albuginea; the processus vaginalis invaginates through this mass. The testis rides behind the enlarging epididymal tail, consistent with epididymal-led descent [2-4] (Fig 1).

What classic experiments really demonstrated

Bergh et al. (1978) cut the gubernaculum at different levels in rats and found that cutting the distal gubernaculum (blocking formation of the processus vaginalis) prevented descent, whereas severing proximally did not, if the scrotal pathway still developed [5]. Contemporary reviews have echoed this reinterpretation and emphasized gubernacular expansion in humans as a key step during the inguino-scrotal phase [2-4].

Molecular drivers: INSL3, androgens, and where the epididymis fits

The refined two-phase paradigm

The canonical two-phase model is transabdominal (placental hCG; INSL3 from fetal Leydig cells) and inguino-scrotal (androgen-dependent) and remains broadly valid [4]. The deeper insight is *where* these signals act:

- Androgen/INSL3 differentiates epididymal smooth muscle/myofibroblasts, enabling motility of the ETU during the inguino-scrotal step [1,4] (Fig 2).

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Evidence that the epididymis is a molecular target

In INSL3-deficient mice, epididymal development is aberrant and the ETU fails to descend properly, implicating epididymal maturation as a functional readout of the INSL3 pathway [3]. This epididymal smooth muscle axis offers a mechanistic bridge between endocrine cues and physical descent [6]. In 2010, we reported impaired FGFR1 expression in the undescended testis of unilateral cryptorchid boys [7]. In addition, decreased FGFR1 protein levels have been found in cryptorchid epididymides of both humans and rodents [7]. These findings support the involvement of FGFR1 in regulating epididymal mesenchyme development. The impaired FGFR1 protein secretion found in underdeveloped mesenchyme in cryptorchid humans and rodents likely contributes to the defective epididymis formation and consequent undescended position. Thus, the observed decrease in FGF expression may result in decreased EGR gene expression, inducing congenital hypogonadotropic hypogonadism and maldescent of the ETU [7].

Reconciling the “gubernacular” and “epididymal-led” views

What the gubernaculum does

The gubernaculum swells, becomes extracellular matrix-rich, and dilates the inguinal canal/processus vaginalis, stabilizing the ETU as it traverses the abdominal wall. Distal interruption (failure of processus formation) blocks descent even if endocrine signaling is intact [5]. Thus, no path means no passage. Our gubernacular definition in accordance with van de Broeck explicitly excludes the conus inguinalis observed in rodents (but missing in humans) as a gubernaculum [8]. Furthermore, we consider the distal part of the gubernaculum a scrotal anlage with different physiological functions in different mammalian species [8].

What “pulls” or “pushes”

Histology does not show a robust fibromuscular cable inserting into the tunica albuginea to pull the testis. Instead, the epididymal tail advances first and, with androgen-primed myoid elements along the epididymal duct and mesentery, the testis follows, cradled by the epididymal curve and mesorchium. In this ETU model, the gubernaculum is the door-opener and the epididymis the pathfinder and mover [1,2].

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Misleading diagrams and the need for histology

A recurring problem has been didactic schemata that paste the gubernaculum directly onto the testis and omit the epididymis. When checked against histology plates, these images do not match reality because the gubernacular fibers blend with epididymal connective tissues and vasal mesentery, and the testis lacks traction footprints one would expect if it were a primary target of a shortening “tendon” [4,9,10,11]. The community should prioritize image-backed anatomy over iconography.

Conclusion

The accumulated molecular, experimental, and anatomical evidence supports an integrated model in which the epididymis leads and the gubernaculum enables. The epididymal tail descends first, the gubernacular mass opens the route distally, and the testis follow as part of an ETU.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding none

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Figure 1. Epididymo-testicular descent in humans. The gubernaculum (G) is proximally attached to the Wolffian duct (WD) and cauda epididymis (E). The mesenchymatous gubernaculum enlarges from the 125 mm crown rump (CR). The epididymis enfolds the testis while descending and pushes the testis down. The caput epididymis pressure induces flattening of the upper testicular pole, particularly at the 350 mm CR. GM, mesonephros; EO, external oblique; K, kidney. [4]

Figure 2. Sagittal section of a heterozygous Insl3 mouse. (a) The testis is located at the bladder neck with developed cauda epididymis. The arrow points towards the gubernacular bulb, where the tip of the gubernaculum is inserted. (b) Immunostaining of the cauda epididymi shows strongly stained smooth muscle arranged in a circular fashion around the epididymal duct (arrow). The tunica albuginea and testicular peritubular connective tissue are also stained. Sagittal section of a homozygous Insl3 male mutant mouse with epididymis and testis located in proximity to the lower kidney pole. The cauda and corpus of the epididymis are underdeveloped. Smooth musculature is absent around the epididymal duct in Insl3 homozygous male mutant mice. [6]

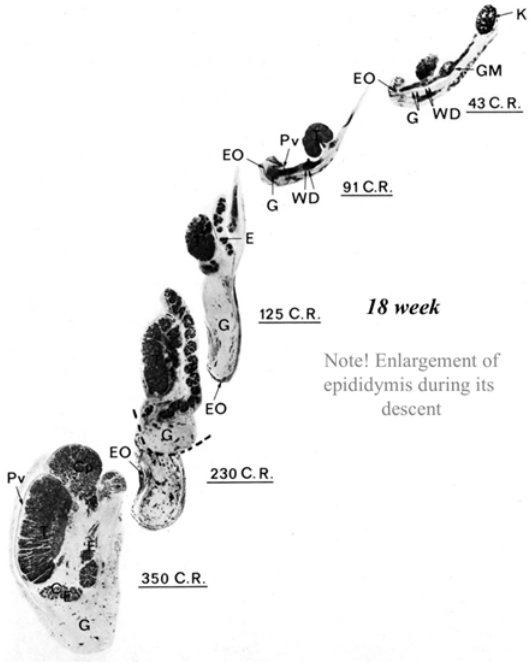

Figure 1.

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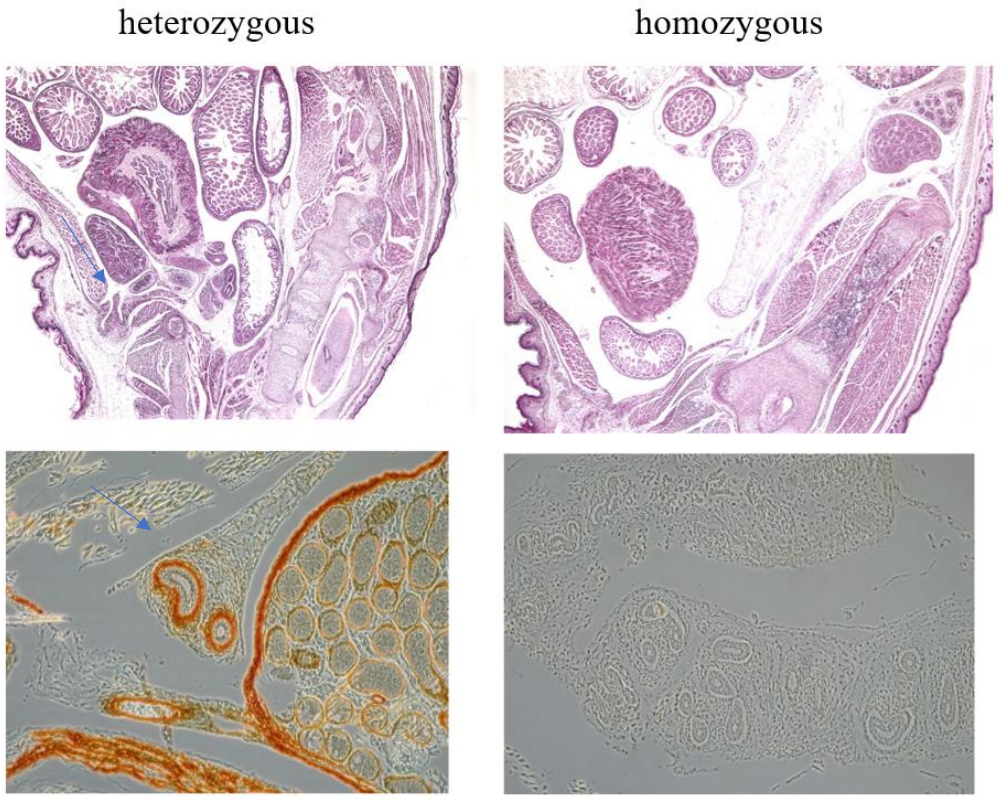

Figure 2.

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Advantages and disadvantages of early orchidopexy

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Abstract

The optimal timing of orchidopexy for cryptorchidism remains debated despite guidelines favoring repair between 6–12 months; this narrative synthesis integrates histologic, endocrine, and clinical outcome data — framed by the symposium transcript from Vassalli Hall — to evaluate the advantages and disadvantages of early surgery. Earlier orchidopexy (≤12 months) is consistently associated with better postoperative testicular growth and germ-cell preservation, with surrogate markers of fertility (germ-cell counts, semen parameters) generally superior to those seen after delayed repair. However, recent series show comparable rates of testicular atrophy and perioperative complications across early and later cohorts in experienced hands. Countervailing considerations include the possibility of intrinsic, irreversible testicular defects that limit benefit, technical and anesthetic challenges in smaller infants, and the risk of unnecessary operations stemming from diagnostic inaccuracy, all compounded by persistent real-world delays that undermine adherence to early-repair guidance. Overall, the weight of evidence supports early orchidopexy — ideally between 6 and 12 months — as the strategy most likely to preserve testicular tissue and enhance future fertility without increasing operative risk, while emphasizing individualized planning for high intra-abdominal testes and the need for standardized outcomes and long-term fertility (live-birth) endpoints to definitively quantify benefit.

Key words Cryptorchidism, early orchidopexy, strategy

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Résumé

Le moment optimal de l'orchidopexie pour le traitement de la cryptorchidie demeure débattu, bien que les recommandations actuelles privilégient une intervention entre 6 et 12 mois ; cette synthèse narrative, intégrant les données histologiques, endocriniennes et cliniques — et s'appuyant sur le discours du symposium de Vassalli Hall — évalue les avantages et les inconvénients d'une chirurgie précoce. Orchidopexie réalisée avant l'âge de 12 mois est systématiquement associée à une meilleure croissance testiculaire postopératoire et à une préservation accrue des cellules germinales, avec des marqueurs substitutifs de fertilité (nombre de cellules germinales, paramètres spermatiques) supérieurs à ceux observés après une correction plus tardive. Cependant, tandis que les taux d'atrophie testiculaire et de complications opératoires demeurent comparables entre les groupes, dans les séries récent menées par des équipes expérimentées ; les arguments contraires incluent la possibilité de lésions testiculaires intrinsèques et irréversibles limitant le bénéfice, les difficultés techniques et anesthésiques propres aux nourrissons, ainsi que le risque d'interventions inutiles liées à des erreurs diagnostiques, le tout aggravé par des retards persistants dans la prise en charge qui compromettent l'application des recommandations ; dans l'ensemble. Les données disponibles soutiennent l'orchidopexie précoce — idéalement entre 6 et 12 mois — comme la stratégie la plus à même de préserver le tissu testiculaire et d'améliorer la fertilité future sans accroître le risque opératoire, tout en soulignant la nécessité d'une approche individualisée pour les testicules intra-abdominaux hauts et d'études à long terme standardisées portant sur la fertilité (naissances vivantes) afin de quantifier de façon définitive le bénéfice.

Mots-clés: Cryptorchidie, orchidopéxie précoce, stratégie

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Introduction: Cryptorchidism, histopathology, and rationale for surgical correction

Definitions, epidemiology, and natural history

Cryptorchidism (undescended testis, UDT) is defined as failure of one or both testes to descend into the scrotum by birth or within early infancy. It is one of the most common congenital anomalies encountered in pediatric urology/andrology, with a birth prevalence generally of about 2–5% in full-term male infants, and higher among preterm infants (estimates up to ~30%). [1]. Many testicles will descend spontaneously in the first few months of life (especially by 3–6 months), after which spontaneous descent is rare. [2]. After 6 months, ongoing spontaneous descent is unusual; thus, persistent cryptorchidism beyond 6–12 months prompts evaluation for surgical correction. [3].

Cryptorchidism may be unilateral or bilateral and may present in different anatomical locations: intra-abdominal, inguinal canal, suprascrotal, or at the external inguinal ring. Nonpalpable testes (often intra-abdominal) may require imaging or laparoscopy to be localized. [2]. Some testes may be retractile (i.e. normally descend into scrotum with manipulation) or ascending (initially descended but later ascend). Distinguishing retractile from true undescended testes is important. [2].

Risk factors for cryptorchidism include prematurity, low birth weight, genetic predisposition, androgen-insensitivity or hormonal defects, environmental endocrine disruptors, and maternal factors. [2].

Left untreated, cryptorchidism is associated with risks including impaired spermatogenesis/fertility, testicular atrophy, increased risk of testicular malignancy, torsion risk, and inguinal hernia [2,4].

Histopathology and progression of testicular degeneration

One of the key rationales for early surgical intervention is the histologic and cellular damage that accrues over time in an undescended testis exposed to higher-than-scrotal temperatures and perhaps abnormal endocrine or paracrine milieu.

- **Germ cell depletion and apoptosis.** Multiple studies have documented a decline in germ cell (including spermatogonia) number and quality over time in undescended testes. After the first year of life, a progressive loss of germ cells may occur, reducing the population of stem spermatogonia and impairing future spermatogenesis. [5].
- **Morphological changes in seminiferous tubules.** In prolonged undescended testes, histologic features may include thickening of the basement membrane, interstitial fibrosis, loss or degeneration of germinal epithelium, Leydig cell changes, and reduced tubular diameter. [2].
- **Impaired hormonal milieu.** The undescended testis may suffer from microenvironmental disturbances, altered temperature regulation, oxidative stress, or local endocrine dysregulation — potentially influencing Leydig cell function, Sertoli cell function, or paracrine support for spermatogenesis. [2].

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- **Irreversibility over time.** The longer the gonadal tissue remains undescended, the more likely that degenerative changes become irreversible, limiting the restorative potential of surgery [6].

These pathophysiologic insights motivate the idea that earlier relocation of the testis to the cooler scrotal environment may halt or partially reverse damage, preserve germ cells, and optimize fertility potential.

Goals and aims of orchidopexy

The aims of orchidopexy can be summarized (and critiqued) as follows:

1. **Prevent or reduce testicular atrophy**
2. **Preserve or improve spermatogenic capacity / fertility potential**
3. **Prevent further deterioration**
4. **Minimize complications (vascular injury, vas deferens damage)**
5. **Reduce long-term risks, e.g. malignancy**
6. **Correct anatomical position for monitoring, maintenance**

In surgical practice, choices of technique (open vs laparoscopic, Fowler-Stephens stages, gubernaculum-sparing, microvascular anastomosis, etc.) and timing must be aligned with those aims and balanced with the risks.

Thus, the question of **timing** — and specifically whether *early* orchidopexy confers net advantage over later surgery — remains central and somewhat unsettled in the literature.

Definition of “early orchidopexy” and guideline recommendations

Before delving into pros and cons, it is essential to define what is meant by “early orchidopexy” and to understand international guideline consensus (or lack thereof) on the timing.

What is “early”?

In the literature, “early” orchidopexy is variably defined but generally refers to relocation of the undescended testis before 12 months of age — often between 6 and 12 months. Some authors push even earlier (before 6 months), though this is less common and not universally accepted. [2,7]. Some comparative studies group operations by age (e.g., ≤12 months vs >12 months) to compare outcomes. [5].

In practice, many centers historically delayed orchidopexy to later infancy, toddler age, or even childhood (e.g. age 2–3 years), but more recent guidelines have increasingly recommended earlier intervention. [3].

Some recent analyses use metrics like *growth percentage ratio (GPR)* to compare testicular growth relative to contralateral testis, stratified by timing of surgery. [1].

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Key guideline recommendations and practice surveys

- The *European Association of Urology / European Society for Pediatric Urology (EAU/ESPU) Guidelines* for management of undescended testes typically recommend orchidopexy **ideally between 6 and 12 months**, and certainly by 18 months of age at latest.
- AUA (American Urological Association) / pediatric urology guidelines tend to similarly favor early referral and surgery (though practices vary). [8].
- Many observational surveys show that actual practice often lags guideline ideal: few orchidopexies are conducted before 12 months of age, even a decade after guidelines exist. For instance, a German national analysis reported only 15% of hospital patients underwent orchidopexy before age 1, and only 5% in private practice did so, even 10 years after guideline publication. [3].
- The study "Are we still too late? Timing of orchidopexy" observed persistently low rates of early orchidopexy, prompting calls for increased awareness and adherence. [3].
- A Greek observational single-center study echoed that guidelines generally recommend corrective surgery by 12 months and no later than 18 months, yet many patients are operated later. [9].
- The "Nordic consensus on treatment of undescended testes" recommends preferably surgical treatment and not in general hormonal treatment between 6 and 12 months of age, or upon The "Nordic consensus on treatment of undescended testes" recommends preferably surgical treatment and not in general hormonal treatment between 6 and 12 months of age, or upon diagnosis. [10].
- The European Society for Pediatric Urology Guidelines state that there is good evidence for early correction of undescended testes preventing potential impairment of fertility and reduce the risk of testicular malignancy. No consensus exists on the various forms of hormonal treatment, which are assessed on an individual basis. [11].

Thus, while there is growing consensus recommending early surgery (preferably before or around 12 months), real-world adherence remains suboptimal, and there remains debate about the exact "optimal" cutoff.

Advantages of early orchidopexy

Here we consider the potential benefits — supported by molecular, histologic, imaging, endocrine, and clinical evidence — of performing orchidopexy at an earlier age.

Preservation of germ cell number and quality

One of the strongest arguments for early surgery is that relocating the gonad earlier limits the ongoing loss of germ cells and may preserve the stem spermatogonial pool:

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- Multiple histologic studies show that germ cell counts decline with age in undescended testes, so earlier relocation might "catch" the testis before irreversible depletion. [2].
- Some studies show that earlier surgery is correlated with less severe germ cell loss and better germ cell maturation indices. For instance, meta-analyses suggest fertility potential may be better in early orchidopexy groups. [6].
- The systematic review and meta-analysis referenced in the literature concluded that although atrophy and complication rates may not differ between early and delayed orchidopexy, early surgery may offer better fertility potential. [6].
- The retrospective study of orchiopexy within 1 year vs later showed that earlier intervention significantly accelerates growth of the undescended testis, using a new metric GPR (growth percentage ratio). The early group had GPR ~2.02 compared to ~1.25 in older groups. [1].

Thus, by decreasing the exposure time of the testis to a suboptimal environment, early surgery might preserve more germ cell reserves and improve the "starting point" for future spermatogenesis.

Improved testicular growth / volume retention

Another measurable objective is preserving testicular size and volume:

- The study from Nature (2017) reported that boys undergoing orchiopexy under 1 year demonstrated significantly higher GPR than those operated later, implying that earlier surgery supports better catch-up growth of the undescended testis relative to the contralateral testis. [1].
- More broadly, a systematic review (2025) on testicular atrophy and growth found that earlier management correlates with better growth outcomes, while delayed surgery is associated with greater risk of volume loss [12].
- The logic is that early repositioning to cooler scrotal environment helps restore more normal physiology, permitting better recovery or continued growth, whereas prolonged delay allows atrophy or fibrosis to set in.

Better fertility / spermatogenesis outcomes

Since the ultimate functional goal is fertility, the question is whether early surgery leads to better adult spermatogenesis:

- Some meta-analyses and systematic reviews suggest that early orchidopexy is associated with increased sperm counts and better semen parameters compared to delayed surgery. [6].
- One review argued that fertility potential "may be better with early orchidopexy." [6].
- In bilateral cryptorchidism, the risk of infertility is highest; early bilateral correction may optimize residual testicular function. [2].

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- Even in unilateral cases, early correction may reduce the "second hit" on the contralateral testis or limit hormonal crosstalk effects. Some authors argue that unilateral cryptorchidism still carries some subtle fertility reduction, so early surgery may mitigate that. [2].

Though the literature is not uniform, the trend across multiple observational and meta-analytic works leans toward better fertility outcomes with earlier surgery.

Prevention of further deterioration and irreversible damage

Early orchidopexy may help arrest or slow the progression of damage:

- It may prevent further germ cell loss, fibrosis, or interstitial changes that accumulate over time, thus preserving tissue structure before irreversible degeneration. [2].
- By intervening earlier, one may mitigate secondary insults due to heat, oxidative stress, vascular compromise, or microenvironmental disturbances.
- Surgeons often reason that "damage may be reversible early but not late," so earlier intervention gives more "room" for recovery. This aligns with Vassalli Hall's emphasis that we want to "prevent further deterioration."

Potential reduction of malignancy risk / earlier surveillance

Although the evidence is less definitive, early orchidopexy might influence long-term risks:

- Undescended testis is a known risk factor for testicular germ-cell tumors (about 4–40× increased relative risk). [4].
- Some epidemiologic data suggest that earlier orchidopexy (before puberty or before age 10) may reduce cancer risk more than later correction. [2].
- Early placement in the scrotum allows for easier surveillance (palpation, ultrasound) and earlier detection of any neoplastic changes.

Psychological, anatomical, and practical benefits

Other practical or secondary benefits include:

- A testis in the scrotum is more accessible for examination, palpation, monitoring, and self-examination in the future.
- Anatomical repositioning at an earlier age may reduce the risk of future torsion or associated hernia.
- Earlier surgery may minimize the period during which the child and family deal with the anxiety or uncertainty of undescended testis.
- From a logistical standpoint, earlier repair may integrate with other pediatric care schedules and prevent backlog or delays in surgical waitlists.

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Disadvantages, risks, and counterarguments of early orchidopexy

While early surgery has many theoretical and empirical advantages, it also carries potential downsides, uncertainties, or trade-offs.

Surgical risks (vascular, vas modeling, anesthesia concerns)

- **Vascular injury and testicular atrophy.** In a small infant, the testicular vessels may be shorter or more delicate; dissection and mobilization carry risk of devascularization or microvascular compromise leading to testicular atrophy. Vassalli Hall himself mentions “damage of the blood vessels” in the transcript.
- **Damage to vas deferens.** The vas and its blood supply may be at higher relative risk in very young patients.
- **Technical difficulty.** The smaller anatomical size, fine tissues, and fragility may make surgery more technically demanding.
- **Anesthesia risk.** Infants under 6 or 12 months may have higher anesthetic risk, though modern pediatric anesthesia has reduced this significantly. [13].
- **Overtreatment / unnecessary surgery.** If spontaneous descent would have occurred (rare beyond 6 months), early intervention might represent overtreatment, though that is less of a concern if one enforces a minimal observation period.

Lack of solid randomized controlled trial evidence and bias

- Much of the literature is observational, retrospective, or heterogeneous in design, limiting the strength of causal inferences.
- Confounding factors (e.g. selection bias, surgical skill, follow-up duration, baseline testis health) may bias results. Vassalli Hall remarks: “all the studies … are biased by one or other way” (transcript).
- There is no universally accepted standard or protocol for measuring outcomes (germ cell counts, testis volume, fertility endpoints) which complicates comparative assessment.

Diminishing returns if testicular pathology is already present

- In some cases, testicular pathology (germ cell loss, fibrosis) may already have occurred prenatally or very early postnatally. Earlier surgery may not reverse such intrinsic damage.
- If the undescended testis is already severely compromised, early orchidopexy may not improve fertility outcomes meaningfully.

Technical limitations and anatomical constraints

- In some nonpalpable or high intra-abdominal testes, surgical techniques (e.g. staged Fowler-Stephens) require more complex planning; doing them very early may limit surgical options or increase risk.

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- The length of the cord, vascular length, and risk of tension or traction injury may be more challenging in infants.
- In cases where the testis is located too far from the internal ring, or vascular length is insufficient, staged procedures or more advanced microvascular techniques may be necessary, and timing must be carefully chosen.

Potential for unnecessary surgery in misdiagnosed cases

- Some children may have initially apparent undescended testis that would descend spontaneously within early months; early surgery may be performed in error or prematurely.
- In the transcript, Vassalli Hall cautions that "a lot of these children are operated, actually, without having an undescended testis." This suggests overdiagnosis or overzealous surgical referral may lead to unnecessary interventions.

Follow-up challenges and long-term proof limitations

- The ultimate proof of fertility is the ability to father a child. Many studies do not have long-term follow-up into adulthood, making it hard to confirm that early surgery yields higher live birth rates.
- Differences in follow-up duration, drop-out rates, and inconsistent outcome measurement hamper comparability.

Synthesis of evidence: what the data says

Atrophy and complication rates

The systematic review and meta-analysis comparing early vs late orchidopexy found that *atrophy* and *complication rates* do not appear significantly different between early and delayed surgery, but fertility potential may trend better with earlier repair. [6]. This suggests that early timing does not increase surgical morbidity, supporting the safety of early intervention.

Testicular growth metrics

As noted above, the study using growth percentage ratio (GPR) showed significantly better post-operative growth when orchidopexy is done under 1 year, compared to older ages. [1]. A more recent review also confirms that earlier management is correlated with better testicular growth. [1,12,14].

These indicate that early orchidopexy confers measurable improvements in testicular volume/size metrics.

Fertility and spermatogenesis outcomes

Literature is less uniform in this domain, but umulative evidence trends toward a benefit:

- Some studies report of improved sperm counts or semen parameters in early orchidopexy groups.

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- The caveat is that many studies are small, retrospective, or vulnerable to selection bias or confounding by surgeon skill, pathology severity, or duration of follow-up.
- Bilateral cryptorchidism remains a high-risk scenario where outcomes are more consistently poor; early correction may at least maximize residual function. [15].
- One study "Fertility potential in adult men treated for uncorrected bilateral..." emphasizes that earlier correction is broadly considered beneficial, although residual impairment may persist. [15].

Ultimately, although early orchidopexy does not guarantee fertility normalization, it likely improves the odds compared to delayed repair.

Practice versus guideline adherence

Despite increasing consensus on early repair, actual practice continues to lag. The German national data (15% in hospital, 5% private before age 1) exemplifies this. [3]. (SpringerLink) Similar patterns have been observed in other countries. [2]. Reasons include delayed referral, lack of awareness, scheduling delays, or resource constraints.

Gaps, controversies, and uncertainty

- The absence of randomized controlled trials comparing early vs delayed surgery limits level I evidence.
- Heterogeneity in study designs, surgical techniques, follow-up, and outcome definitions muddy conclusions.
- The threshold of how "early" is optimal remains debated (e.g., before 6 months vs within 12 months).
- In high intra-abdominal testes or cases requiring complex surgical strategies, the timing question becomes even more nuanced.
- The intrinsic baseline pathology (prenatal damage, genetic defects) may limit the benefit of timing.
- Long-term fertility endpoints (live births) are rarely studied, and many studies rely on surrogate markers (germ cell counts, semen parameters).

Practical recommendations and "balanced view"

Given the above, what practical guidance or balanced stance can be advanced?

Suggested optimal timing

- Based on current evidence and guideline consensus, orchidopexy is ideally performed **between 6 and 12 months of age**, while delaying beyond 18 months is suboptimal.
- If surgical logistics permit, earlier in that window (closer to 6 months) may yield slightly better growth or germ cell preservation, but trade-offs (anesthesia, surgical risk) must be considered.

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- For very high intra-abdominal testes or when complex staged procedures are anticipated, timing may be individualized.

Decision framework considerations

When deciding timing, the surgeon/endocrinologist should weigh:

1. **Baseline status of the testis** (palpable vs nonpalpable, vascular length, location)
2. **Surgical risk and feasibility** (can the testis be mobilized safely at that age?)
3. **Anesthetic risk and institutional capability**
4. **Potential for spontaneous descent** (typically negligible beyond 6 months)
5. **Surveillance logistics and referral delays**
6. **Familial preferences and psychosocial factors**

Technique and surgical strategy alignment

- Early surgery does not mandate inferior techniques; experienced centers can use microdissection, microsurgical techniques, and minimal-invasive approaches to reduce risks.
- In cases where early relocation is not feasible anatomically (e.g. short vessels), a staged or modified approach (e.g. Fowler-Stephens, Shehata technique) might be appropriate, even if at slight delay. [2].
- Preservation of the gubernaculum, use of laparoscopy, and gentle handling can mitigate surgical risk even in younger infants. [5].

Monitoring and long-term follow-up

- Rigorous follow-up, including serial ultrasound, hormonal evaluation, sperm parameter analysis (when of age), and clinical surveillance is essential to assess outcomes.
- Central registries or prospective cohorts would help strengthen evidence.
- Longitudinal tracking into adult fertility outcomes, including paternity data, is crucial to better define ultimate benefit.

Acknowledging uncertainty and individualization

- Surgeons and clinicians must acknowledge that not every child will achieve “normal” fertility despite early surgery, due to intrinsic damage or unknown factors.
- Timing decisions should be made in multidisciplinary settings (surgeon, endocrinologist, pediatric urologist) with shared decision-making with families.
- In the face of resource constraints or scheduling delays, earlier prioritization of cryptorchid cases is justified based on potential benefit.

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Integration with the transcript and thematic commentary

The transcript from Vassalli Hall offers several relevant reflections and significant facts, which we can overlay on this evidence-based discussion:

- He underscores the fundamental aims of orchidopexy (prevent atrophy, preserve fertility, avoid damage) and the challenges of surgical technique, vascular risk, and vas injury.
- His rhetorical question, "When should we do the operation?" mirrors the crux of this paper.
- He notes that historically, surgeries were done later (e.g. around 10 years of age in the 1950s), but gradually timing has shifted toward earlier ages (e.g. 6 months).
- He cautions that many operations are performed even without a true undescended testis, indicating diagnostic overreach—this warns us to be prudent in patient selection.
- He emphasizes that literature comparing early vs late is sparse and biased, and that endocrine and molecular biologists must help answer when is optimal (rather than surgeons operating blindly).
- He expresses uncertainty: "Is it conclusive that delayed surgery … equals decreased fertility potential? We don't know."
- He also raises the question of reversibility and inherent defects: if a testis is already pathologic, early relocation may not suffice.

These points highlight that while clinical and surgical mats exist, the truly firm answers remain elusive, reinforcing that a cautious but proactive stance is optimal.

Limitations, gaps, and future research directions

To drive forward progress in the field, future research should address:

- **Prospective, ideally randomized, trials** of early vs delayed orchidopexy (though ethical/logistical barriers exist)
- **Standardization of outcomes**, including germ cell counts, testicular volume measures, semen parameters, and long-term fertility endpoints
- **Long-term follow-up cohorts** tracking live birth/paternity, hormonal function, malignancy incidence
- **Molecular and biomarker studies** to identify which testes are more salvageable — e.g. genomic, proteomic markers, testicular microenvironment signatures
- **Imaging and noninvasive assessments** predicting viability and "reserve" preoperatively

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- **Refinement of surgical techniques** (microsurgery, vascular-sparing, novel traction-based approaches) tailored to younger patients
- **Health services and implementation studies** to reduce delays and increase guideline adherence
- **Cost-benefit, risk-benefit modeling** incorporating anesthesia risk, surgical resource allocation, long-term outcomes

By bridging molecular, endocrine, and surgical perspectives (exactly in line with the symposium thematic), future consensus on optimal timing may emerge.

Conclusion

In sum, the balance of evidence and expert consensus currently leans toward **early orchidopexy**—ideally between 6 and 12 months of age—as the prudent strategy to maximize testicular preservation, mitigate germ cell loss, enhance testicular growth, and improve fertility potential, all without clear added surgical risk. The disadvantages or risks are real but appear manageable in expert hands, and do not appear to outweigh the potential benefits in most cases. That said, definitive proof remains elusive due to limitations in existing studies, and individualization of timing based on anatomical and clinical factors remains necessary.

At the same time, as the transcript emphasizes, we must not be complacent: the literature remains thin, real-world practices lag guidelines, and deeper, multidisciplinary research is needed. Surgeons, endocrinologists, and molecular biologists must collaborate to refine the timing question, discover biomarkers of salvageability, and track long-term functional outcomes.

Given his stature and experience, Professor Zachariou's participation in this symposium offers a meaningful bridge between surgical tradition and evolving molecular-endocrine frontier. His insights, combined with the emerging evidence, can help guide more definitive recommendations and stimulate dialogue on this enduring question.

I look forward to any feedback, suggestions, or additional focal points (e.g. more molecular detail, region-specific data) that you would like me to expand.

Declaration Section

a) Ethics Approval and Consent to Participate Investigations were carried out in accordance 326 with the Declaration of Helsinki of 1975, revised in 2008.

b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

e) Funding No financial conflicts.

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Benefits and drawbacks of hormonal treatment/orchidopexy

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Abstract

Cryptorchidism is a symptom with multifactorial etiologies that range from endocrine signaling failure and epididymo-gubernacular mechanics to primary testicular dysgenesis. In contemporary practice, surgical orchidolyse and orchidopexy is standardized and widely successful at placing the testis in its normal scrotal environment; however, hormonal therapy remains controversial. This paper synthesizes translational and clinical evidence—including guideline positions, randomized trials, meta-analyses, long-term cohort data, and biopsy-based histology—to appraise the benefits and drawbacks of (1) primary hormonal treatment and (2) adjunctive hormonal therapy after successful orchidopexy, alongside (3) the benefits and limitations of orchidopexy itself. Special attention is given to fertility-relevant endpoints (Ad spermatogonia, inhibin B, semen analysis) and real-world outcomes such as paternity. We conclude that primary hormonal therapy should **not** be used to induce descent, but carefully selected, low-dose, intermittent GnRH analog therapy **after** orchidopexy may benefit germ-cell maturation in boys with congenital true cryptorchidism and boys with bilateral ascending cryptorchidism and proven histological low fertility potential, whereas orchidopexy—performed expertly and early (ideally ≤12–18 months)—remains the gold standard for durable scrotal positioning, improved testicular growth trajectories, and facilitation of surveillance. The decision to offer adjuvant hormonal therapy should be individualized, biopsy-informed when feasible, and framed by shared decision-making about uncertain long-term fertility gains.

Key words Cryptorchidism hormonal treatment orchidopexy

Résumé

La cryptorchidie est un symptôme à étiologies multiples, incluant des défauts de signalisation endocrinienne, des anomalies épididymo-gubernaculaires et une dysgénésie testiculaire primaire. Si l'orchidolyse et l'orchidopexie sont aujourd'hui standardisées et efficaces pour rétablir la position scrotale, l'utilisation d'un traitement hormonal demeure controversée. Cette synthèse évalue, à partir de données translationnelles et cliniques (recommandations, essais randomisés, méta-analyses, cohortes à long terme, histologie testiculaire), les bénéfices et limites de (1) la thérapie hormonale primaire, (2) la thérapie hormonale adjuvante après orchidopexie réussie, et (3) l'orchidopexie elle-même. Une

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attention particulière est portée aux critères de fertilité (spermatogonies Ad, inhibine B, analyses de sperme) ainsi qu'aux résultats réels tels que la paternité. Les données disponibles indiquent que la thérapie hormonale primaire ne doit pas être utilisée pour induire la descente, mais qu'un traitement adjuvant par agoniste de la GnRH, à faible dose et de façon intermittente, peut améliorer la maturation germinale chez des garçons soigneusement sélectionnés présentant un faible potentiel de fertilité. L'orchidopexie précoce (≤12–18 mois) demeure toutefois la référence pour garantir une position scrotale durable, des trajectoires de croissance testiculaire favorables et une surveillance adéquate. L'indication d'une thérapie hormonale adjuvante doit être individualisée, idéalement éclairée par la biopsie, et discutée dans un cadre de décision partagée en raison de bénéfices fertiles à long terme encore incertains.

Mots-clés: traitement hormonal, cryptorchidie orchidopexie

Introduction and standpoint

As pediatric urologists, we try to "mimic nature," restoring a testis to its intended scrotal niche and supporting its maturation. Yet cryptorchidism is not a single disease: it is a **final common phenotype** produced by varied upstream defects—endocrine (e.g., impaired mini-puberty), mechanical (e.g., epididymo-gubernacular interface and processus vaginalis dynamics), and primary testicular production errors (intrinsic dysgenesis) [1,2]. In such heterogeneity, no single therapy will cure all.

In 2025, two pillars define management:

- **Surgical orchidolyse and orchidopexy** (open or laparoscopic, depending on location) remains the gold standard to achieve and maintain scrotal position, with well-established techniques and high success rates when performed by experienced surgeons.
- **Hormonal therapy** (hCG, GnRH or LHRH analogs) is not supported as **primary** descent-inducing therapy in true undescended testes, but there is emerging and debated evidence that **adjunctive** low-dose, intermittent GnRH analog treatment following successful orchidopexy may promote germ-cell maturation and potentially improve later fertility in selected boys [1–5].

This review articulates the benefits and drawbacks of these approaches, aligns them with guidelines, and maps them to outcomes that matter for families—especially **fertility and paternity**—while weaving in pragmatic insights from clinical practice (e.g., diagnosis and timing) as presented in the symposium session.

Pathophysiologic frame: why treatment choice matters

Multifactorial origins

The pathogenesis of cryptorchidism involves:

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- **Endocrine signaling** (HPT axis, "mini-puberty," androgens' effects on downstream tissues),
- **Mechanical guidance** (epididymal development, gubernacular swelling, patent processus vaginalis), and
- **Primary gonadal dysgenesis** (intrinsic germ-cell deficits) [1,2].

Because etiologies differ, the **response to hormones** and the **value of surgery alone** differ across subgroups. Recognizing this, modern guidelines place primary emphasis on **timely orchidopexy** and **selective** use of hormones for fertility preservation rather than descent induction [1–3].

What endpoints should we value?

Beyond "the scrotal position of the testis," relevant endpoints include:

- **Histology** (presence of Ad spermatogonia; Nistal score) as a proxy for future spermatogenesis;
- **Serum markers** (inhibin B, FSH/LH, testosterone) that reflect Sertoli/Leydig function;
- **Semen parameters** in adulthood;
- **Paternity**, acknowledging it is an imperfect, culturally and behaviorally confounded endpoint but ultimately family-important [1–3,6–9].

Primary hormonal therapy to induce testicular descent: benefits and drawbacks

Evidence of limited efficacy for descent

Multiple systematic reviews and meta-analyses—Cochrane and others—have shown **modest** and **inconsistent** descent rates with hCG or GnRH when used as **primary therapy**, with **frequent re-ascent** and **no durable benefit** compared with surgery [3–5]. Consequently, major guidelines advise **against** routine use of hormonal therapy to induce descent in true undescended testes [1–3].

- The AUA guideline (2014, reiterated in later overviews) concludes that **providers should not use hormonal therapy** to induce testicular descent owing to low response rates and lack of sustained efficacy [2].
- The EAU/ESPU guidance similarly **does not recommend** medical treatment to induce descent, but leaves room for **post-surgical** fertility-oriented regimens in selected boys [1].

Benefit: Rare cases (particularly canalicular testes close to the scrotum) may transiently descend with GnRH, possibly avoiding an operation in the short term.
Drawbacks: Low sustained success; risk of **re-ascent**; and with **hCG**, androgenic adverse

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effects (scrotal pigmentation, penile growth, behavioral changes), pain, and concerns about **germ-cell apoptosis** in some experimental and clinical reports [3–5,10,11].

Net: Do not use primary hormones to induce descent in true cryptorchidism. Proceed to timely orchidopexy.

Safety profile of hormones

- **hCG:** Androgenic side-effects are common; some studies suggest increased germ-cell apoptosis and potential harm to future spermatogenesis, especially with higher/longer dosing [10,11].
- **GnRH/LHRH (e.g., buserelin):** Nasal low-dose regimens are generally well tolerated, with mild local effects; systemic adverse events are uncommon at intermittent, low doses used post-orchidopexy in published series [4,5,9].

Surgical orchidolysis and orchidopexy: benefits and drawbacks

What orchidolysis and orchidopexy reliably delivers

- **Anatomic success:** Experienced pediatric urologists achieve high, durable scrotal positioning, particularly when employing complete cremasteric dissection, division of the processus vaginalis high at the internal ring, and a tension-free dartos pouch placement ("subdartos")—critical steps to prevent recurrence [1,2].
- **Testicular environment:** Scrotal relocation represents a lower than the core body temperature, favoring germ-cell survival compared to retained abdominal/inguinal position.
- **Growth/Surveillance:** Earlier surgery aligns with better testicular growth trajectories and enables palpation for tumor surveillance later in life [1–3].
- **Biopsy opportunity:** In intra-operative contexts, biopsy permits risk stratification (Ad spermatogonia presence, Nistal score) to guide adjuvant decisions [6–9].

Timing matters

The consensus trend has shifted **earlier**: perform surgery by **12 months** and no later than **18 months** (corrected for prematurity), recognizing that many boys who are undescended at 6 months will not achieve spontaneous descent thereafter [1–3]. Earlier orchidopexy correlates with higher rates of favorable histology and better long-term function in population analyses and guideline syntheses [1–3].

Limits of surgery

- **Intrinsic dysgenesis:** Some testes are histologically poor regardless of position; surgery alone cannot convert a Sertoli-only or severely depleted germ-cell compartment into normal spermatogenesis.

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- **Ascending (acquired) testis:** Even testes documented as scrotal in early childhood can ascend later, often with a patent processus vaginalis; vigilance and school-age examination programs remain important [12]. There are 2 peak moments for higher chance to develop ascending testis in the development of the child: 6-7 years and 11-12 years of age. It has been shown that fertility potential can be compromised in the ascending testis at this age, and this although the testis was previously documented in the scrotum.

- **Fertility not guaranteed:** Orchidopexy improves the odds but is not synonymous with normal semen or paternity—especially in **bilateral** disease [6–9].

Post-orchidopexy GnRH/LHRH therapy aimed at fertility: benefits and drawbacks

Rationale: rescuing mini-puberty–linked germ-cell maturation

Ad (dark) spermatogonia emergence during mini-puberty is a key determinant of later sperm output. Histologic studies associate **absence of Ad spermatogonia** in cryptorchid testes with **poor adult semen** and subfertility. This has led to the hypothesis (spearheaded by Hadžiiselimović and collaborators) that **low-dose, intermittent GnRH** after the testis is in the scrotum may **re-trigger** or **amplify** HPT-axis signaling to promote germ-cell maturation (Ad transformation), benefiting future fertility [4,6–9].

What the data show

- **Histology:** Several series report **increases in germ-cell counts** or **Ad spermatogonia** after **nasal buserelin** (e.g., 10 µg every other day for 6 months) in boys at high infertility risk on biopsy, compared with surgery alone [6–9]. Some cohorts documented durable histologic gains at follow-up biopsy approximately two years later [6–9].

- **Hormones:** Improvements or favorable trends in **inhibin B** (Sertoli function) and **LH/testosterone** have been observed in long-term follow-ups of boys treated with adjunctive GnRH versus controls, though sample sizes are modest and selection biases possible [8,9].

- **Adult semen/paternity:** Adult-age outcomes remain limited by loss to follow-up and cohort size. Available studies suggest **possible** semen quality advantages in treated boys, particularly those with bilateral disease and high-risk histology, but **conclusive** paternity benefits are not yet robustly demonstrated across independent centers [7–9].

Benefit: In **selected** high-risk boys (bilateral cryptorchidism, poor histology), post-orchidopexy low-dose GnRH may **improve germ-cell maturation markers** and **hormonal milieu**, plausibly translating into better semen in adulthood.
Drawbacks: The evidence base is **not uniform**; randomized, adequately powered, multi-center trials with **adult** endpoints are sparse. Access to **buserelin** or equivalent formulations varies by country; treatment necessitates months-long adherence. Families should be counseled that benefits are **probable but not guaranteed**.

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Fertility and paternity after cryptorchidism: what should we tell families?

Unilateral vs bilateral

- **Unilateral cryptorchidism**: Paternity rates approach the general population (≈90–94%) in many series when treated appropriately, although subtle semen abnormalities can occur [12].
- **Bilateral cryptorchidism**: Paternity is **reduced** (≈60% range in classic cohorts), even with surgery; fertility counselling is essential .

These figures, cited in guideline summaries and classic cohorts, underline the **heterogeneity** of outcomes and the importance of early, expert care [1–3,].

Translating histology to outcomes

Biopsy-based **risk stratification** (e.g., Nistal scoring, Ad-spermatogonia presence) correlates with later **inhibin B** and semen quality in several cohorts, including Leuven series where routine biopsies informed longitudinal counseling and, when feasible, adjunctive therapy [8,9]. Where pathology expertise is available, biopsy adds **prognostic power** and refines shared decision-making.

Practical diagnosis and selection pitfalls (why some "failures" are preventable)

Although this paper focuses on treatment, outcomes are sensitive to **accurate diagnosis** and **selection**, themes repeatedly emphasized in our clinical program:

- **Differentiate** retractile testes from true undescended; the cross-legged "frog" position and patience (2 minutes) help the cremasteric fibers relax, improving palpation accuracy. Over-diagnosis leads to unnecessary surgery; under-diagnosis delays needed care [1,2].
- **Avoid routine imaging** (US/CT/MRI) before referral for palpable testes; imaging seldom changes management and may mislead. Ultrasound can aid in **non-palpable** or very obese infants but has modest sensitivity/specificity; **diagnostic laparoscopy** is the gold standard when the testis is truly non-palpable [2,13].
- **Recognize ascending (acquired) testis:** School-age peaks in orchidopexy reflect acquired ascent; many of these boys have a patent processus vaginalis at surgery [12]. Vigilant pediatric screening programs matter.

Sound diagnosis ensures we apply the **right therapy** to the **right child** at the **right time**—the foundational benefit that underpins all downstream gains.

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Putting it together: individualized, multifactorial care

A contemporary algorithm

1. **Confirm the phenotype**
    - Palpable vs non-palpable; true undescended vs retractile; consider syndromic contexts (e.g., hypospadias, DSD). Avoid non-actionable imaging [2,13].
2. **Time surgery appropriately**
    - Plan orchidopexy by **12 months** (no later than **18 months**), earlier if spontaneous descent seems unlikely after 6 months corrected age [1–3]. Use meticulous technique (complete cremasteric dissection, high ligation of processus vaginalis, careful spermatic vessel handling, tension-free subdartos placement).
3. **Consider biopsy when it will change management**
    - Especially in **bilateral** disease and/or late presenters, biopsy (if local pathology expertise exists) can stratify infertility risk (Ad spermatogonia) and guide **adjuvant GnRH** discussions [6–9].
4. **Offer adjunctive low-dose, intermittent GnRH after successful orchidopexy selectively**
    - Particularly in **bilateral** undescended testes with **high-risk histology**; counsel families about potential benefits (germ-cell maturation, hormonal markers) vs uncertainties (adult paternity) and practicalities (drug access, adherence) [4,6–9].
5. **Long-term follow-up**
    - Growth, position, testicular consistency; adolescent counseling; adult fertility assessment when appropriate.

Where the field should go

- **Multi-center RCTs** powered for adult outcomes (semen/paternity) to definitively test post-orchidopexy GnRH strategies;
- **Biomarker refinement** (beyond biopsy): non-invasive surrogates of germ-cell health would democratize selection;
- **Mechanistic work** on HPT-axis programming and epididymo-gubernacular mechanics to uncover new adjuncts (e.g., CGRP-pathway modulators).

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Benefits and drawbacks—succinct comparative summary

Strategy	Key benefits	Key drawbacks / limits
Primary hormonal therapy (hCG, GnRH) to induce descent	Rare short-term descent in selected low canalicular testes	Low durable success, high re-ascent; hCG side-effects and possible germ-cell harm; **not recommended by guidelines** [1–5,10,11]
Orchidopexy (early, expert)	High durable scrotal position; improved environment; surveillance; enables biopsy-guided risk stratification	Cannot correct intrinsic dysgenesis; fertility not guaranteed, especially bilateral; requires anesthesia and surgical expertise [1–3,6–9]
Adjunctive low-dose, intermittent GnRH after successful orchidopexy	In selected high-risk boys, improves germ-cell markers (Ad spermatogonia), inhibin B trends; plausible semen benefit	Evidence heterogeneous; adult paternity benefit not conclusively proven; limited access to buserelin in some regions; months-long adherence [4,6–9,]

Conclusions

- **Surgery first:** For true undescended testes, **orchidopexy**—performed **early** and **expertly**—remains the cornerstone with the most certain benefits.
- **No to primary hormones for descent purpose:** Routine **hormonal induction of descent** is **not** recommended; benefits are small and transient; risks and re-ascent are real.
- **Yes, consider post-surgical GnRH selectively:** After successful orchidopexy, **low-dose, intermittent GnRH analog therapy(6 months)** may confer **fertility-relevant** advantages in **biopsy-defined high-risk** boys, particularly with bilateral disease. Families should be informed of **potential gains** and **current uncertainties**.
- **Measure what matters:** Where possible, anchor decisions in **histology** (Ad-spermatogonia, Nistal score) and track **inhibin B** and **long-term semen**. Continue to pursue **adult** endpoints, including paternity.
- **Get the basics right:** Accurate clinical diagnosis (no-touch patience, frog-leg positioning), avoidance of unnecessary imaging, and recognition of **ascending testis** will reduce overtreatment and undertreatment alike.

The overarching message is pragmatic: **multifactorial condition, multifactorial care**. We should keep our scalpel sharp, our endocrine lens focused, and our humility intact as we match the **right** patient to the **right** combination at the **right** time.

Declaration Section

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b) Consent for publication Not applicable

c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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Is the current guidance for clinical management of cryptorchidism based on the latest research findings?

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Abstract

International guidance on cryptorchidism has been shaped by three reference documents: the Nordic consensus (2007), the American Urological Association (AUA) guideline (2014), and the periodically updated European Association of Urology/European Society for Paediatric Urology (EAU/ESPU) guidelines, which were most recently revised in 2024. These statements agree regarding early orchidopexy (generally before 12–18 months of age), avoidance of routine imaging before specialist referral, and laparoscopy for non-palpable testicles. Clinical studies demonstrate that surgery alone—even early and expertly performed surgery—does not fully restore male reproductive potential in 50% of cryptorchid boys belonging to the high infertility risk (HIR) group. This patient group suffers hypogonadotropic hypogonadism, such that the logical treatment approach includes hormonal replacement. Since the drafting of the main reference guidelines, emerging translational evidence has refined our understanding of the biology of mini-puberty, the prognostic value of Ad spermatogonia, and the molecular/epigenetic landscape that underpins impaired germ-cell maturation in cryptorchidism. The present review appraises which aspects of contemporary guidance remain robust, which recommendations lag behind new data, and where pragmatic multidisciplinary updates are likely warranted. It concludes with targeted proposals aimed at aligning future guidance with the latest science, while preserving the feasibility of implementation across varied health systems.

Key words. Cryptorchidism, treatment, surgery, hormones

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Résumé

Les recommandations internationales concernant la cryptorchidie reposent sur trois documents de référence : le consensus nordique (2007), les recommandations de l'Association américaine d'urologie (AUA) (2014) et les recommandations périodiquement mises à jour de l'Association européenne d'urologie/Société européenne d'urologie pédiatrique (EAU/ESPU), dont la dernière révision date de 2024. Ces recommandations s'accordent sur l'orchidopexie précoce (généralement avant l'âge de 12 à 18 mois), l'absence de recours systématique à l'imagerie avant consultation spécialisée et la laparoscopie en cas de testicule non palpable. Des études cliniques démontrent que la chirurgie seule, même précoce et réalisée par un expert, ne permet pas de rétablir pleinement la fertilité masculine chez 50 % des garçons cryptorchides appartenant au groupe à haut risque d'infertilité (HRI). Ces patients souffrent d'hypogonadisme hypogonadotrope, ce qui justifie l'instauration d'un traitement hormonal substitutif. Depuis l'élaboration des principales recommandations de référence, les données translationnelles émergentes ont affiné notre compréhension de la biologie de la mini-puberté, de la valeur pronostique des spermatogonies Ad et du paysage moléculaire/épigénétique sous-jacent à l'altération de la maturation des cellules germinales dans la cryptorchidie. La présente revue évalue quels aspects des recommandations actuelles restent pertinents, quelles recommandations sont en retard par rapport aux nouvelles données et où Des mises à jour pragmatiques et multidisciplinaires sont probablement nécessaires. Le document se conclut par des propositions ciblées visant à aligner les futures recommandations sur les données scientifiques les plus récentes, tout en préservant la faisabilité de leur mise en œuvre dans divers systèmes de santé.

Mots clés : cryptorchidie, traitement, chirurgie, hormones

Introduction: why re-open "settled" questions?

In pediatric urology, guidelines have the power to shape referrals, parental expectations, intervention timing, and how success is measured [1–3]. While the "gold-standard" surgery, orchidopexy, is standardized and safe, it is not curative in cases where cryptorchidism is more than a positional problem. Even with appropriate surgery, long-term outcomes, including adult fertility and paternity, remain suboptimal for men with previously undescended testicles [4–10]. New data regarding the biology of mini-puberty and germ-cell transformation challenge the notion that testicular repositioning alone is sufficient to ensure future reproductive potential in a substantial subset of patients [11–14].

This review examines whether current guidance is fully aligned with the most recent evidence. The three most influential guidance documents are summarized. Next, specific domains are reviewed—including diagnosis, imaging, timing, surgical technique, and the potentially crucial role of hormonal therapy—using recent studies and translational work

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presented at *The 5th International Andrology Symposium — Cryptorchidism: Molecular Biology meets Endocrinology and Surgery*. Finally, areas where the current recommendations should be refined are identified and denoted.

Brief summaries of the reference guidance

Nordic consensus (2007)

The Nordic statement was seminal in consolidating early surgery (ideally before 1 year of age), deemphasizing imaging, and recommending laparoscopy for non-palpable testicles [1]. It reflects skepticism towards the use of hormonal therapy to induce testicular descent. Many of its surgical and diagnostic positions have aged well; however, some of its recommendations are now outdated. Notably, it has not been updated to reflect the latest results regarding restoration of fertility potential, i.e. spermiograms.

AUA guideline (2014)

The AUA reinforces early orchidopexy, and discourages routine imaging before specialist evaluation, since ultrasonography has poor sensitivity for non-palpable testicles and rarely changes management strategy [2]. Critically, the AUA advises against administering hormonal therapy to induce descent, due to low response and lack of durable efficacy [2, 15, 16]. However, this position is contradicted by the results of long-term follow-up studies, with Höcht et al. reporting that 52% of testicles remain descended at 10 years after hormonal treatment [17], and Waldschmidt et al. showing that 50.5–67% of testicle remain descended at 5–7 years after treatment [18].

EAU/ESPU guidelines (most recently updated in 2024)

The recommendations of the EAU/ESPU mirror those of the AUA with regards to diagnostic and surgical fundamentals, imaging, and timelines, with surgery ideally performed between 6–12 months of age, and not later than 18 months of age [3]. The distinctive position of the EAU/ESPU guidelines is to *consider* low-dose GnRH analogues as an adjunct for fertility preservation in bilateral cases, *after* successful orchidopexy, with acknowledgement of the evidence showing improved markers of germ-cell maturation (e.g. Ad spermatogonia) in carefully phenotyped cohorts [3, 17–22].

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Where guidance is strong, and should remain unchanged

The primacy of clinical examination and the retractile trap

In all statements, the hands-on exam is rightfully placed at the center of diagnosis and triage. Distinguishing a retractile testicle from a truly undescended testicle carries profound consequences: the former requires only observation, not surgery or hormones; while the latter benefits from timely surgery to correct its positioning [2, 3, 23–26]. Patience, proper positioning (e.g. cross-legged “frog” position), and allowing the cremaster to fatigue are pragmatic and evidence-concordant practices that reduce misclassification.

Imaging seldom helps before referral

The AUA and EAU/ESPU guidelines discourage the use of ultrasound for initial evaluation, since this method exhibits poor sensitivity and specificity for non-palpable testicles, and recommend that these patients should be referred for examination by an experienced pediatric surgeon or urologist [2, 3, 27, 28]. Data from the Choosing Wisely® initiative suggest that inappropriate imaging remains common despite guidance, underscoring the need for education and system-level reminders [29].

Laparoscopy for non-palpable testicles

Diagnostic laparoscopy is the gold standard, offering immediate therapeutic paths—including orchidopexy, one- vs two-stage Fowler–Stephens, or confirmation of testicular absence [2, 3, 23, 30–32]. No new data have overturned this approach.

Where guidance lags the evidence, and what to change

Early surgery is better than late

Although surgery cannot guarantee a positive final fertility outcome, all major guidelines recommend that orchidopexy be performed within the first year of life (ideally 6–12 months of age), and certainly before 18 months of age, with the aim of minimizing acquired histological damage [1–3, 16, 33].

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The biology of mini-puberty and Ad spermatogonia: surgery alone is insufficient for 50–70% of boys

Mini-puberty, during the first 3–6 months of life, is characterized by transient rises of GnRH/LH/FSH and testosterone, which drive the critical transition of gonocytes to type A dark (Ad) spermatogonia—i.e. the human spermatogonial stem-cell pool [5, 11–14]. Failure of this transition predicts severe oligospermia/azoospermia later in life, despite technically successful orchidopexy; azoospermia development is observed in 32% of patients with bilateral undescended testicles, and 10% with unilateral undescended testicles [5–11, 13, 34]. Studies in multiple cohorts demonstrate that an absence or paucity of Ad spermatogonia in early biopsies is linked to poor adult sperm counts in cases of cryptorchidism [5, 6, 10–13, 34]. In other words, a correct *position* of the testicle is necessary, but *biology* determines fertility potential.

Evidence-concordant niche for hormonal therapy

Some guidance states that hormonal therapy to *induce testicular descent* is ineffective, or not durably effective, and should not be used as primary treatment [1–3, 15, 16]. However, the EAU/ESPU guidelines include a carefully worded allowance to *consider* low-dose intermittent treatment with GnRH analogues after successful orchidopexy in *selected* boys, with bilateral disease and histologic signs of impaired germ-cell maturation. This recommendation is aligned with translational and clinical signals showing improved markers (e.g. increased Ad spermatogonia and biomarkers of Sertoli cell function) and possibly better long-term semen parameters in some cohorts [3, 17–22]. In one study, early surgery showed no positive impact on these parameters, as the sperm quality of the surgical group did not differ from the sperm results of untreated boys, and these authors concluded that hormonal therapy is advisable for all patients with cryptorchidism, independently from the surgical option to promote testicular descent to the scrotum [22]. A recent narrative synthesis and small prospective series consistently demonstrate these benefits, while emphasizing the need for careful selection and standardized protocols [17–22]. Best results were obtained with Pergoveris,(LH/FSH 75/150 IU) therapy; all 20 bilateral intraabdominal cryptorchid testes descended to the scrotal position [35].

Biopsy: when, where, and for whom?

Routine biopsy of all cryptorchid testes should be performed at the time of orchidopexy to identify patients who require subsequent hormonal treatment. Biopsy analysis by a trained pathologist can provide powerful prognostic information, e.g., Ad spermatogonia counts [5, 6, 10–14, 36]. Notably, there are real-world barriers to routine biopsy—including inconsistent pathology expertise, lack of standardized reporting, and system incentives that discourage

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the practice. However, none of these barriers constitute a scientific reason to avoid biopsy where it can improve case management.

Implication for guidance

It is increasingly indefensible to treat every boy with cryptorchidism as if surgery alone will normalize risk. Guidance must be updated to acknowledge the prognostic role of Ad spermatogonia in cryptorchidism, regardless of whether it is unilateral or bilateral; as well as to describe structured pathways for centers with the capability to phenotype risk (biopsy and expert histology) and to offer adjuvant time-limited GnRH analogue therapy after orchidopexy for the patients at highest risk [3, 5–14].

Practical domains

Clinical examination: get the basics right, every time

- Continue: Performing methodical calm palpation with attention to child comfort, frog-leg position, and adequate time; clear documentation; distinguishing retractile vs truly undescended testicles; counseling parents about surveillance for ascent.
- Refine: Teach and credential primary-care and neonatal examiners; embed testicle position into standardized newborn documentation, which is still too often missing [23–25].
- Stop: Scheduling surgery for retractile testicles; ordering ultrasound before referral to a specialist (pediatric surgeon or urologist), except in unusual scenarios (e.g. obesity obscuring a clearly palpable canalicular testicle) [2, 3, 27–29].

Imaging

- Continue: Not performing routine ultrasound, CT, or MRI before referral.
- Refine: Selectively use ultrasound (e.g. equivocal canalicular mass in a very large child) with awareness of its limits; do *not* delay referral or surgery for imaging [2, 3, 27, 28].
- Stop: Using imaging “for reassurance”—data show that it wastes resources and doesn’t reduce downstream surgeries [29].

Laparoscopy and surgical technique

- Continue: Performing diagnostic laparoscopy for non-palpable testicles; meticulous mobilization; high ligation of processus vaginalis; creation of a superficial dartos pouch for fixation; two-stage Fowler–Stephens when required [2, 3, 23, 30–32].

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- Refine: Recognize and manage anatomic variants (e.g. short vessels, intra-abdominal position, and epididymal/gubernacular anomalies) that influence strategy; document vessel number and collateral patterns—fetal data suggest that multiple arterial supplies are common and clinically relevant for planning [16, 24].
- Stop: Making deviations from standardized steps (e.g. failing to deal with a patent processus vaginalis) that increase re-ascent risk [2, 3, 23, 24, 30–32].

Hormonal therapy

- Acknowledge: That Ad spermatogonia have a prognostic role regarding fertility potential in cryptorchidism [5, 6, 10–14, 19–21].
- Describe: Structured pathways for centers having the capability to phenotype infertility risk (testicular biopsy and expert histology).
- Implement: Adjuvant time-limited GnRH analogue therapy after orchidopexy in patients with HIR.

Follow-up and outcomes

- Continue: Performing clinical checks for re-ascent and testicular volume asymmetry.
- Refine: Where feasible, transition programs should include offers of semen analysis at adulthood, with counseling that paternity (the outcome that matters most to families) can often be achieved even when semen parameters are suboptimal; facilitate assisted reproduction referrals when indicated [4–14, 19–21].
- Stop: Equating a “good operation” with “normal fertility”—communicate honestly about bilateral disease.

Conclusions

The current guidance regarding cryptorchidism remains strong in terms of its surgical core—which includes early expert orchidopexy, minimal pre-referral imaging, and laparoscopy for non-palpable testicles. However, it lags in translating a decade of progress in developmental and molecular andrology into actionable and equitable recommendations for fertility preservation in *HIR cryptorchid* boys. The EAU/ESPU guidelines include a cautious allowance for post-orchidopexy treatment with GnRH analogues in such cases, which is congruent with the best available translational signal. The AUA could converge with this narrow risk-stratified use, while continuing to discourage hormone therapy for inducing testicular descent. An interdisciplinary update—co-authored by experts in pediatric surgery/urology and pediatric endocrinology—would harmonize global practice, reduce unwarranted imaging and operations and, most importantly, give families evidence-based and realistic paths to parenthood. Overall, the next edition of guidance should retain its surgical backbone, while adding a measured endocrine “limb”, which will constitute a potentially decisive addition for boys whose risk is determined by biology, rather than just anatomy.

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c) Availability of data and supporting material Not applicable

d) Competing interests Author/s declare that they have no competing interests

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